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RENAL PHOSPHATASE

THE CORRELATION BETWEEN THE FUNCTIONAL ACTIVITY OF THE RENAL TUBULE AND ITS PHOSPHATASE CONTENT

HARRY A. WILMER, M.D.

MINNEAPOLIS

A specific histochemical stain for the demonstration of alkaline phosphatase in ordinary histologic slides was independently described by Gomori [1] and Takamatsu [2] in 1939. Later a similar technic was devised by Gomori [3] to show activity of acid phosphatase in tissue sections. Both acid and alkaline phosphatase are present in the kidney. Little if anything is known concerning the specific biologic role of acid phosphatase, but there is considerable evidence that alkaline phosphatase is concerned in the reabsorption of sugar and possibly in the metabolism of carbohydrates. Alkaline phosphatase in the kidney is not concerned with calcification. Grosser and Husler [4] in 1912 reported activity of alkaline phosphatase in a number of mammalian tissues; they found the greatest concentration in the kidney and the intestine. Histochemical studies by Gomori [5] revealed that the enzyme in the kidney is limited to the proximal convoluted tubules and a varying portion of the ascending loop of Henle, while in the intestine it is limited to the luminal portion of the mucosal epithelium. This picture is constant and uniform. Acid phosphatase in the kidney is variable in intensity and in situation.

It was quickly pointed out that the position of phosphatase in the proximal convoluted tubules and in the intestine was added evidence for the participation of the enzyme in the reabsorption of sugar.[6] In fact, it was because of the great phosphatase activity of the kidney and the intestine that a connection was sought between phosphorylation, and dephosphorylation by phosphatase in absorption. The reabsorption of dextrose is limited to the proximal convoluted segment of the renal tubules.[7] It has been suggested that phosphatase dephosphorylates hexosephosphates formed in the reabsorption of carbohydrates. No function has been ascribed to the enzyme present in the loops of Henle. For that matter, alkaline phosphatase is present in detectable amounts in almost all mammalian tissues, especially calcifying cartilage, bone, liver, lung, bladder and capillary endothelium. This study is concerned with renal alkaline phosphatase in the epithelium of the proximal convoluted tubules.

Brain and Kay [8] first pointed out that phosphatase activity is a sensitive indication of the functional state of the kidney. These authors found that the phosphatase activity of fetal and very young kidney is considerably less than that of older renal tissue. In patients with chronic nephritis dying of uremia the phosphatase content of the kidney is markedly diminished, on the average to one-sixth the normal value. When death is not due to nephritis but degenerative conditions are found in the kidney, the phosphatase content of the renal tissue is much reduced. In patients with severe infective processes there is shown some diminution of renal phosphatase activity but much less than in patients with nephritis and degenerative renal lesions. Moreover, these authors found that nephritis induced with uranium nitrate in rabbits caused a reduction in phosphatase activity of about one half.

Umeno,[9] Tomikawa [10] and Ohno [11] confirmed and extended the observation of reduced phosphatase activity in experimental nephritis, the last author noting the increased urinary excretion of phosphatase, which apparently came from the kidney. All of these studies, depending on extracting whole renal cortex, gave no clue to the precise intracellular enzyme changes.

From the Department of Pathology, University of Minnesota.

1. Gomori, G.: Proc. Soc. Exper. Biol. & Med. **42**:23, 1939.
2. Takamatsu, H.: Tr. Jap. Path. Soc. **29**:492, 1939.
3. Gomori, G.: Arch. Path. **32**:189, 1941.
4. Grosser, P., and Husler, J.: Biochem. Ztschr. **39**:1, 1912.
5. Gomori, G.: J. Cell. & Comp. Physiol. **17**:71, 1941.
6. Lipmann, F., in Nord, F. F., and Werkman, C. H.: Advances in Enzymology and Related Subjects, New York, Interscience Publishers, Inc., 1941, vol. 1, p. 99.
7. White, H. L., and Schmidt, F. O.: Am. J. Physiol. **76**:483, 1926.
8. Brain, R. T., and Kay, H. D.: Biochem. J. **21**: 1104, 1927.
9. Umeno, M.: Biochem. Ztschr. **231**:328 and 334, 1931.
10. Tomikawa, S.: Arb. a. d. anat. Inst., Kyoto (Abt. 3) **100**:62, 1932.
11. Ohno, K.: J. Biochem. **33**:35, 1941.

More recent work employing a histochemical technic has shown that phosphatase, although reduced, is still abundant in kidneys severely injured by nephrotoxic drugs,[12] but instead of being sharply limited to the luminal portion of the cells it is more diffusely spread through the cell. Phlorhizin, which prevents reabsorption of sugar, does not inactivate phosphatase,[13] and therefore its action is concerned with interruption of phosphorylation and not with dephosphorylation. When the ureter is ligated, most of the renal phosphatase disappears after five to seven days of hydronephrosis.[12b] Giliberti,[14] employing biochemical methods, was unable to detect any phosphatase in 5 hydronephrotic kidneys after forty-eight hours of ureteral obstruction, while noting that at twenty-four hours the activity was approximately half of the normal value. By the use of the delicate histochemical method we have always been able to demonstrate small quantities of phosphatase in hydronephrotic kidneys up to an interval of sixty-three days in a series now totaling 64 rabbits, cats and rats. The hydronephrotic kidney is able to reabsorb sugar since the concentration of dextrose in the hydronephrotic sac is less than that in the glomerular filtrate or in the blood plasma.[15] The small amount of phosphatase persisting is probably essential for the reabsorption of the sugar in a glomerular filtrate, which in all likelihood is greatly reduced.

In hydronephrosis there is severe tubular dysfunction as evidenced by the inability of the kidney to concentrate urine and by the fact that the hydronephrotic kidney is protected against uranium nitrate and mercury bichloride poisoning.[12b] In hydronephrosis phosphatase rapidly disappears, yet in experimental lesions it remains active, although quantitatively reduced. Because of the unique sensitivity of the phosphatase content to a functional disturbance it was surmised that tubular function might be correlated with phosphatase content.

With this in mind, the phosphatase activity of the renal tubules was demonstrated following complete and following partial ureteral obstruction and after release of such obstruction; also following vascular episodes, such as temporary arrest of renal circulation and permanent ischemia. It was investigated in rabbits with spontaneous interstitial nephritis and in a number of animals and fish, notably the aglomerular toadfish, Opsanus tau, whose tubules were found to be devoid of this enzyme. The metabolism in the kidney which was not subjected to operative interference and in the hydronephrotic kidney was determined on slices of renal tissue by the Warburg manometeric method, dextrose substrate being used.

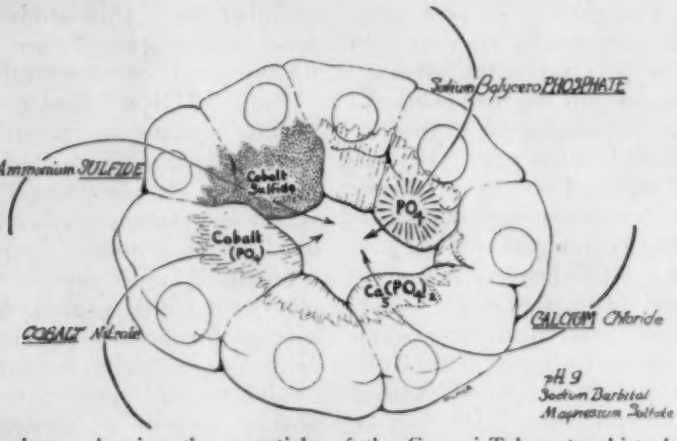

Fig. 1.—Composite schema showing the essentials of the Gomori-Takamatsu histochemical technic for the demonstration of alkaline phosphatase. Enzyme present in the luminal portion of the renal tubule is indicated by light shading. The enzyme hydrolyzes sodium betaglycerophosphate with liberation of inorganic phosphate, which is precipitated by calcium ions present in the incubating solution. The colorless calcium phosphate is converted to black cobalt sulfide in subsequent solutions.

EXPERIMENTAL PROCEDURE

Phosphatase activity was determined by the Gomori technic with slight technical modifications.[16] Since phosphatase is not inactivated by alcoholic fixation or embedding in paraffin, microscopic sections on slides still retain their enzymatic activity. A solution of formaldehyde will inactivate the phosphatase. The essentials of this histochemical technic have been incor-

12. (*a*) Hepler, O. E.; Simonds, J. P., and Gurley, H.: Proc. Soc. Exper. Biol. & Med. **44**:221, 1940. (*b*) Wilmer, H. A.: J. Exper. Med. **78**:225, 1943.

13. Kritzler, R. A., and Gutman, A. B.: Am. J. Physiol. **134**:94, 1941.

14. Giliberti, P.: Arch. ital. di urol. **15**:217, 1938.

15. Achard, C.: Arch. franco-belges de chir. **31**:359, 1928.

16. Kabat, E. A., and Furth, J.: Am. J. Path. **17**:303, 1941.

porated in a schematic representation (fig. 1). A mounted section of tissue is incubated in a solution of sodium betaglycerophosphate and calcium chloride buffered to p_H 9.1 with barbital sodium and containing magnesium sulfate. The magnesium ion is a specific activator of alkaline phosphatase, although its presence is not essential for satisfactory preparations. The enzyme, indicated by the lightly shaded areas in the drawing, hydrolyzes the sodium betaglycerophosphate substrate with liberation of inorganic phosphate at the site of enzyme activity. The calcium ions precipitate an insoluble calcium phosphate. After incubation for two hours the preparation is stained for inorganic phosphate. It is placed in a cobalt sulfide solution which converts the calcium phosphate to colorless cobalt phosphate. This in turn is transformed into the black cobalt sulfide by immersing the slide in a solution of dilute yellow ammonium sulfide. The enzymatic activity is now revealed by the black or deep brown cobalt sulfide compound, and the slide is counterstained with hematoxylin and eosin. The phosphatase activity of the kidney is judged on the average density, the size and the distribution of the dark granules in three or four sections taken from different parts of the kidney. Duplicate control sections are carried through the same steps with the exception that sodium betaglycerophosphate is omitted from the incubating solution. This reveals any preformed calcium phosphate. Adjacent renal slices were fixed in Helly's solution (a mixture of mercury bichloride and solution of formaldehyde U. S. P.) and stained with hematoxylin and eosin.

TABLE 1.—*Disappearance of Phosphatase from the Hydronephrotic Kidney*

Rabbit	Duration of Hydronephrosis, Days	Comment	Renal Phosphatase Activity: Right (No Surgical Interference)	Renal Phosphatase Activity: Left (Hydronephrotic)
R- 1	16 hr.	.....	++++	+++
R- 2	2 days	.....	++++	++++
R- 3	3	.....	++++	+++
R- 4	3	.....	++++	+++
R- 5	4	.....	+++	++
R- 6	6	.....	++++	++
R- 7	7	.....	++++	+
R- 8	8	Interstitial nephritis	++++	+
R- 9	8	Interstitial nephritis	++++	++
R-10	10	Interstitial nephritis	++++	+++
R-11	10	.....	++++	Trace
R-12	11	Interstitial nephritis	++++	+
R-13	11	.....	++++	+
R-14	12	.....	++++	++
R-15	13	.....	++++	+
R-16	16	Severe tubular atrophy	+++	Trace
R-17	22	.....	++++	Trace
R-18	24	.....	++++	Trace
R-19	28	.....	++++	+
R-20	62	.....	+++	Trace
R-21	33	Papilla ligated	++++	+

As in the previous study [12b] the left ureters of the rabbits were doubly ligated and severed and the animals allowed to live for intervals of sixteen hours to sixty-three days thereafter. Twenty adult rabbits weighing from 1.5 to 2.8 kilograms were used. Anesthesia was induced by intravenous injection of pentothal sodium, and sterile surgical precautions were employed. Animals were killed by injecting 20 cc. of air intravenously or by breaking the neck with a quick blow. Four of these animals had severe spontaneous interstitial nephritis at postmortem examination. In order to produce hydronephrosis without concomitant pelvic dilation, an attempt was made to ligate the single intrapelvic papilla in 4 rabbits, but in only 1 was the procedure satisfactory (table 1).

Partial ureteral obstruction was produced in 7 rabbits as follows: A wire 0.85 mm. in diameter was placed parallel to the ureter about 2 cm. beyond the ureteropelvic junction. The ureter was tied against the wire with a cotton thread, care being taken not to damage it. The wire was then withdrawn, leaving the tie in place. A high grade partial ureteral obstruction resulted. The rabbits were killed at intervals of nine to thirty days. In an attempt to see whether phosphatase would reappear after the release of the obstruction, 3 rabbits were subjected to laparotomy at nine, fifteen and twenty-eight days, respectively; a single small biopsy specimen was taken for study of the phosphatase; the obstructing tie was then removed and the incision closed. These animals were killed ten days after the operation. One of these animals was found to have complete obstruction at the time of death, but the other 2 had no demonstrable obstruction. Since only one small biopsy specimen was available for comparison, it was extremely difficult to judge whether or not phosphatase activity had increased. These findings are therefore not included in the table. Although it seemed that there was some increase in enzyme activity following the release of the obstructed ureter, this fact was not clearly demonstrated (table 2).

TABLE 2.—*Disappearance of Phosphatase After Partial Ureteral Obstruction*

Rabbit	Duration of Hydronephrosis, Days	Renal Phosphatase Activity: Right (No Surgical Interference)	Renal Phosphatase Activity: Left (Hydronephrotic)
R-22	9	++++	++++*
R-23	14	++++	+
R-24	15	++++	Trace*
R-25	17	++++	++
R-26	20	++++	++
R-27	28	++++	++*
R-28	30	++++	+

* A biopsy specimen was taken.

The cat's glomeruli reveal histochemically intense phosphatase activity.[5] It was of interest to know whether or not the enzyme in the glomeruli would decrease or disappear in hydronephrosis. The left ureters of 6 cats were doubly ligated and severed, and the animals were allowed to live for intervals of from one to twenty-six days. Phosphatase preparations showed rather erratic results, although the enzyme in the glomerulus always persisted even though the tubular phosphatase disappeared. Sometimes no tubular phosphatase was revealed in the kidney with the ureter unobstructed. It was not possible to come to any definite conclusion on this series of animals other than that histochemically the enzyme in the tubule behaved differently from that in the glomerulus.

Using biochemical extracts, Kinard and Chanutin [17] showed that phosphatase rapidly disappears from the kidney after complete ligation of the renal pedicle. In view of the relative disappearance of the enzyme from the hydronephrotic kidney it was considered that phosphatase might be sensitive to the vascular changes associated with hydronephrosis, especially the reduced

17. Kinard, F. W., and Chanutin, A.: J. Biol. Chem. **103**:461, 1933.

blood flow[18] and the resulting renal ischemia.[19] Ligature of the intrapelvic papilla does not interfere with the renal blood flow as does the distention of the pelvis in hydronephrosis. This experiment will be described subsequently. Additional evidence of the effect of renal ischemia on the phosphatase was obtained from the following experiments:

Seven rabbits were subjected to complete constriction of the left renal artery for periods of thirty minutes to one hour and were killed after intervals of eighteen hours to ten days. Through a lateral incision the left renal artery was carefully dissected from the other structures of the pedicle, and a cotton thread was slipped under it and brought to the outside of the wound. The two ends of the thread were clamped with a hemostat, which was placed across the midline of the rabbit's body, the weight of the instrument pulling up on the vessel and occluding it. The kidney immediately became a cyanotic dusky purple; the peritoneum and the skin were clamped together without disturbing the tension or the direction of the cotton thread. At fifteen or twenty minute intervals the kidney was inspected to be sure that the renal artery remained occluded. At the end of the desired interval of time the cotton thread was cut and the kidney observed until its circulation was completely reestablished. In 2 animals the renal vein was ligated and the animals allowed to live for short intervals of time, and in another rabbit the renal vein was constricted for one hour.

In 2 rabbits unilateral renal ischemia was produced by constriction of the left renal artery according to Drury's technic.[20] A wire 0.85 mm. in diameter was placed alongside the renal artery and tied with a cotton thread without damaging the wall of the artery, the lumen of which was partially constricted when the wire was removed. The right kidney was not touched. Blood pressures were taken daily for three days prior to operation and every five to seven days thereafter. In both animals hypertension developed after the operation, but in the first animal it was transient, and at postmortem examination this animal was found to have a necrotic left kidney. In the other animal the hypertension was persistent, and at postmortem examination the left kidney was small, weighing 7.5 Gm., compared with 10.7 Gm. for the kidney with unaltered circulation. Blood pressures were determined with a clinical sphygmomanometer placed around the abdomen and a stethoscope placed over the aorta according to the technic described by McGregor,[21] who reported that the average of 1,120 readings of blood pressure on 84 rabbits was 125 systolic and 90 diastolic. A persistent level in excess of 160 systolic and 100 diastolic was considered hypertension (table 3). In general, the phosphatase activity of the kidney subjected to ischemia was definitely although moderately reduced. Except in 2 rabbits (R-31, R-35) the decrease was not striking.

A miscellaneous group of normal mammals, reptiles, birds and fish were killed and their kidneys studied for alkaline phosphatase activity. Living specimens of the toadfish—an aglomerular marine teleost—were obtained at the John G. Shedd Aquarium in Chicago through the cooperation of Director Walter H. Chute. They had been kept in captivity for several years. They were killed at the aquarium and the kidneys immediately removed by me. All of the 6 toadfish obtained failed to show phosphatase in their tubules. To verify this unexpected observation Director Walter H. Chute sent another toadfish to the laboratory of the department of pathology of the University of Minnesota by express. The fish arrived alive and in good condition, was killed immediately and likewise revealed no tubular phosphatase. Fresh marine fish were not available; however, fresh bass, carp and northern pike were obtained from Minnesota lakes in November by the Minnesota State Game and Fish Commission of the State Department of Conservation. Wild turtles and a salamander were obtained in the same month (table 4). Although the tubules of 1 turtle failed to give a positive phosphatase reaction, those of 4 others showed the enzyme. The tubules of 1 salamander and 1 turtle revealed no renal phosphatase, but the significance of these single observations is obscure. The chicken was found to have the enzyme in its glomeruli. With these exceptions all kidneys examined had phosphatase in the proximal convoluted tubules and sometimes in the loop of Henle but in no other parts of the nephron.

TABLE 3.—*Phosphatase Activity of Kidneys Subjected to Ischemia*

Rabbit	Experimental Procedure	Duration of Post-operative Life	Renal Phosphatase Activity: Right (No Constriction of Renal Vein)	Renal Phosphatase Activity: Left (Ischemic)
R-29	Renal artery constricted 30 min.	18 hr.	++++	++++
R-30	Renal artery constricted 45 min.	20 hr.	++++	+++
R-31	Renal artery constricted 1 hr.	22 hr.	++++	0
R-32	Renal artery constricted 1 hr.	20 hr.	++++	+++
R-33	Renal artery constricted 1 hr.	48 hr.	++++	+++
R-34	Renal artery constricted 1 hr.	33 hr.	++++	+++
R-35	Renal artery constricted 1 hr.	10 days	++++	Trace
R-36	Drury operation (no hypertension)	19 days	++++	+ (necrotic)
R-36b	Drury operation (hypertension)	25 days	++++	+++
R-37	Renal vein constricted 1 hr.	24 hr.	+++	++
R-38	Renal vein ligated	24 hr.	++	++
R-39	Renal vein ligated	48 hr.	++++	+++

Phosphorylation with formation of hexosephosphates is of great importance in the metabolism of carbohydrates. Phosphatases may play a part in intracellular metabolism in the kidney as well as in the reabsorption of sugars. With the disappearance of phosphatase from the hydronephrotic kidney it was of interest to determine whether there was also a reduction in metabolism, even though the two factors might not be directly related.

In 17 rabbits the left ureter was doubly ligated and severed. After intervals of hydronephrosis of from two to sixty-three days the animals were killed and the oxygen consumption of the renal cortex determined on tissue slices by the usual Barcroft-Warburg manometric method (table 5). The experiments were run at 37.5 C. and p_H 7.4. Potassium hydroxide was placed in the inner cup of the flask to absorb the carbon dioxide. Slices of renal cortex taken from several portions of both the hydronephrotic and the unaltered kidney

18. Levy, S. E.; Mason, M. F.; Harrison, T. R., and Blalock, A.: Surgery **1**:238, 1937.

19. Megibow, R. S.; Katz, L. N., and Rodbard, S.: Am. J. M. Sc. **204**:340, 1942.

20. Drury, D. R.: J. Exper. Med. **68**:693, 1938.

21. McGregor, L.: Arch. Path. **5**:630, 1928.

were run simultaneously in triplicate sets and averaged. The thickness of the slices was about 0.4 mm., and a total dry weight of from 7 to 17 mg. was used in each flask. They were suspended in 2.9 cc. of Locke's solution and phosphate buffer, and 0.1 cc. of 0.16-molar dextrose. The cortical tissues of 8 rabbits were run in an atmosphere of oxygen, and those of 9 rabbits in an atmosphere of air. Each determination at a different interval of hydronephrosis was essentially a separate experiment. Since only a single animal was studied at each interval of hydronephrosis the oxygen quotient (or respiration) for the period given cannot be expressed with any degree of certainty. Nevertheless, the oxygen quotient of the unobstructed and that of the hydronephrotic kidney of the same animal at any interval may be compared without hesitancy and a general trend seen. That is to say, the attempt was not to establish precisely the oxygen quotients of the two kidneys at the intervals studied, but to determine whether or not there was a significant difference between the two kidneys in the same animal. At all periods after six days of ureteral obstruction there was a relatively great difference in oxygen consumption (or respiration) between the normal and the hydronephrotic kidney in an atmosphere of oxygen. These animals had received an intravenous anesthetic dose of pentothal sodium one hour before being killed. At the time of death the anesthesia was extremely light.

TABLE 4.—*Phosphatase Activity of the Kidneys of Miscellaneous Animals*

Animal	Number of Animals	Scientific Name	Renal Phosphatase Activity: Tubules	Renal Phosphatase Activity: Nontubular Elements
Toadfish	7	Opsanus tau	0	Lymphoid cells +
Bass	1	Micropterus salmoides	+	0
Carp	1	Cyprinus carpio	+	Intertubular endothelium +
Goldfish	5	Carassius auratus	+	0
Salamander	1	Ambystoma maculatum	0	0
Northern pike	1	Esox lucius	+	0
Chicken	3	Gallus domesticus	+	Glomeruli +
Hog	1	Sus scrofa	+	0
Cotton rat	2	Sigmodon hispidus	+	0
Duck	1	(White pekin duck)	+	0
Turtle	2	Clemmys guttata	+	Intertubular endothelium +
Turtle	2	Clemmys guttata	+	0
Turtle	1	Chelydra serpentina	0	0

COMMENT

Significance of Renal Phosphatase.—The alkaline phosphatase of the kidney has a molecular weight between 6,000 and 10,000.[22] It is possible to inactivate either the acid or the alkaline renal phosphatase and retain the activity of the other,[23] and it is possible to extract one without extracting the other. Perlman and Ferry[24] found that the alkaline and acid phosphatase enzymes are associated with two distinct protein fractions. The acid phosphatase, with an optimum activity near p_H 5, is a water-soluble protein; the alkaline phosphatase, with an optimum activity near p_H 9, is a water-insoluble, salt-soluble globulin. This may be highly significant in understanding how two similar enzymes present in the same cell can be active at rather extreme opposite ends of the p_H range. Spek[25] pointed out that it is not correct to speak of the p_H of the living cell without realizing the hydrogen ion activity of the different protoplasmic components and of the colloid bearers to which the enzymes may be bound. According to Spek, the reaction of the dispersed phase of the protoplasm can deviate considerably from p_H 7, and in the cells which he studied a variation of from 5 to 8 was found by delicate micromethods. On the other hand, the dispersion medium of protoplasmic colloid appeared to be approximately neutral.

Phosphorylation, Dephosphorylation and Phosphatase.—A schematic representation of the reabsorption of dextrose molecules through the renal tubule cells would involve phosphorylation and dephosphorylation (fig. 2). While this schema more than likely may prove to be incorrect in detail, it at least has the advantage of helping in orientation. The oxidation-reduction metabolic processes of the cell are represented in the metabolic generator. Adenosine polyphosphate—which is almost a universal cellular constituent—is indicated by the symbol Ad~P~P~P attached to the generator. Other organic compounds, such as pyrophosphates, may be phosphate carriers. The energy produced, perhaps in the course of aerobic glycolysis, is converted

TABLE 5.—*Metabolism of the Hydronephrotic Kidney: Respiration of the Renal Cortex*

Rabbit	Duration of Hydronephrosis, Days	Oxygen Quotient of Right (Unobstructed) Kidney: 1st Hr.	Oxygen Quotient of Right (Unobstructed) Kidney: 2d Hr.	Oxygen Quotient of Left (Hydronephrotic) Kidney: 1st Hr.	Oxygen Quotient of Left (Hydronephrotic) Kidney: 2d Hr.
Oxygen Atmosphere					
R-40	3	11.1	9.2	11.4	9.7
R-41	6	16.5	14.6	16.9	12.9
R-42	11	19.5	14.1	9.3	8.1
R-43	16	13.0	10.4	7.8	5.1
R-44	23	14.9	12.9	5.6	5.1
R-45*	28	14.5	12.2	10.3	8.7
R-46	37	15.2	13.0	8.6	7.4
R-47	63	17.3	11.1	7.2	6.5
Air Atmosphere					
R-48	2	6.1	5.9	6.3	5.9
R-49	3	6.4	6.4	5.6	5.2
R-50	4	5.6	5.8	5.0	5.3
R-51	6	6.6	6.3	5.7	5.5
R-52	8	6.8	6.4	6.3	5.9
R-53	10	5.9	6.2	5.1	4.6
R-54	15	5.7	5.6	5.2	5.2
R-55	30	8.2	6.6	5.2	3.8
R-56	40	8.3	8.0	5.9	4.0

	1st hr.	2d hr.
Control in air atmosphere	7.0	6.5
Average 10 normal kidneys	6.5	5.6
Average 2 solitary kidneys		(unilateral nephrectomy 3 weeks prior)

* This rabbit was pregnant.

22. Albers, H., and Albers, E.: Ztschr. f. physiol. Chem. **232**:165, 1935.

23. Bamann, E., and Diederichs, K.: Ber ü. d. chem. Gesellsch. **67**:2019, 1934.

24. Perlman, G. E., and Ferry, R. M.: J. Biol. Chem. **142**:513, 1942.

25. Spek, J.: Ergebn. d. Enzymforsch. **6**:1, 1937.

into energy-rich phosphate bonds. That is, available energy is placed in the bonds between the phosphate radicals and the adenosine molecule in a manner comparable to that in creatine phosphate in the muscle. The energy in this linkage is between 9,000 and 11,000 calories, compared with 3,000 calories in the ordinary phosphate ester bond. The tendency to break this energy-rich bond is much greater than the ester linkage; i. e., there is small affinity. With the breaking of this bond there is energy available for metabolic work or for the capture of the dextrose molecule from the urine in the lumens of the tubules. The reader is referred to Lipmann's [6] fascinating paper on energy-rich phosphate bonds. Phosphorylation of the dextrose molecule consists of its union with inorganic phosphate, forming a hexosephosphate. Subsequently the hexosephosphate is dephosphorylated by phosphatase with the liberation of inorganic phosphate and the deliverance of the simple sugar into the blood stream. This process presumably occurs in the luminal portion of the protoplasm.

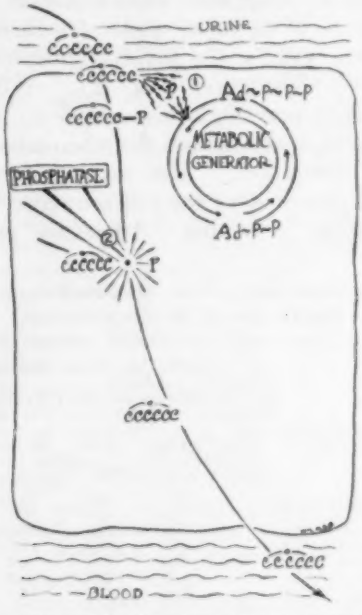

Fig. 2.—Schematic drawing of the hypothetic steps in the transflux of the dextrose molecule through the cells of the renal tubule. Energy-rich phosphate bonds are generated in the metabolic generator for utilization of oxidation-reduction energy. Adenosine polyphosphate yields phosphate to the hexose molecule, forming hexosephosphate, which is subsequently dephosphorylated by phosphatase. Both of these steps probably occur in the luminal portion of the cells.

Lundsgaard [26] proposed that active reabsorption of dextrose from the intestine involved esterification in cells of the absorbing membrane and that a similar process occurred in the renal tubules; he observed that phlorhizin by blocking phosphorylation prevented reabsorption in the intestine and the kidney. Later experiments with the isolated mammalian kidney seemed to have induced him to abandon this theory.[27] Extracts of kidney reveal abundant oxidative phosphorylation [28] and Kalckar [29] reported that this phosphorylation was strongly inhibited by phlorhizin, thus reviving Lundsgaard's original hypothesis.

Presumably the first product of phosphorylation is hexosemonophosphate,[30] a specific substrate of the phosphatases. If the phosphorylation in renal cortex is allowed to progress, the accumulated ester is fructose-diphosphoric ester. The fact that the accumulated ester is a non-oxidized ester makes it unlikely that it is an intermediate product of carbohydrate metabolism, according to Kalckar,[31] but suggests that esterification is related to the reabsorption of dextrose in the tubule. Transfer of phosphate from adenyl pyrophosphate to dextrose might be a step in phosphorylation of dextrose.[31]

Phlorhizin has an inhibitory effect on acid phosphatase but not on alkaline phosphatase [32] and therefore does not interfere with dephosphorylation. Phlorhizin does not inhibit the metabolism of excised renal tissue [33] and inhibits phosphorylation to a much greater extent than consumption of oxygen.[28]

Eichholtz, Robison and Brull [34] suggested that inorganic phosphate in the urine originated from phosphoric esters of the plasma by phosphatase hydrolysis, but Brain, Kay and Marshall [35] showed that urinary phosphates come from inorganic plasma phosphates and concluded that there is probably a renal threshold for excretion of phosphate. This observation does not necessarily mean that renal phosphatase might not play a part in the excretion if the temporary formation of hexosephosphates in the tubules is an intermediate stage in the excretion of phosphate.[36]

26. Lundsgaard, E.: Biochem. Ztschr. **264**:221, 1933.

27. Lundsgaard, E.: Skandinav. Arch. f. Physiol. **72**:265, 1935.

28. Kalckar, H.: Enzymologia **2**:47, 1937.

29. Kalckar, H.: Skandinav. Arch. f. Physiol. **77**:46, 1937.

30. Colowick, S. P.; Welch, M. S., and Cori, C. F.: J. Biol. Chem. **133**:359, 1940.

31. Kalckar, H.: Biochem. J. **33**:631, 1939.

32. Walker, A. M., and Hudson, C. L.: Am. J. Physiol. **118**:130, 1937.

33. Shorr, E.; Loebel, R. O., and Richardson, H. B.: J. Biol. Chem. **86**:529, 1930.

34. Eichholtz, F.; Robison, R., and Brull, L.: Proc. Roy. Soc., London, s.B **99**:91, 1925.

35. Brain, R. T.; Kay, H. D., and Marshall, P. G.: Biochem. J. **22**:628, 1928.

36. Bodansky, A.: J. Biol. Chem. **104**:473, 1934.

The proximal convoluted tubule is capable of actively reabsorbing phosphate.[32] Ekehorn [37] reviewed the evidence for reabsorption of phosphate and sugar in the heart-lung-kidney preparation and concluded that the kidney might handle inorganic phosphate as a threshold substance similar to dextrose, and although the reabsorption was not quantitative it could occur to a high degree.

Pathologic Physiology of the Renal Tubules and Their Phosphatase Content.—Partial ureteral obstruction produces a reduction of tubular phosphatase content but not as quickly as does complete obstruction. Although phosphatase probably reappears after release of a completely or partially obstructed ureter, this fact has not been clearly established.

Assuming that renal phosphatase is essential for the reabsorption of sugar and that a minimum concentration of the enzyme is necessary for proper tubular functioning, one concludes that the hydronephrotic kidney is unable to reabsorb the quantities of sugar which a normal kidney would be called on to withdraw from the glomerular filtrate. The dextrose threshold has its origin in the fact that the tubules can reabsorb sugar up to some constant maximum quantity per unit of time, and any excess passes into the urine. This is the dextrose T_m, or the maximum amount of sugar capable of being reabsorbed in milligrams per minute. If phosphatase is a limiting factor in this function, the dextrose T_m should be reduced in the hydronephrotic kidney.

The vascular changes associated with hydronephrosis were considered possible etiologic factors in the disappearance of phosphatase. To eliminate the stretching, tearing and compression of the vessels of the renal pelvis, as well as the effects of pelvic distention on the vascular system in the parenchyma, the intrapelvic papilla of a rabbit was partially tied, and the animal was allowed to live for one month. When the papilla was being tied, a small portion of it was torn, leaving some of the collecting ducts unobstructed. This resulted in rapid atrophy, probably following dilation of the tubular elements whose collecting tubules were blocked. These atrophic tubules were devoid of phosphatase. However, the proximal convoluted tubules of the nephrons still functioning—presumably those which had not been blocked—showed phosphatase activity characteristic of the normal tubules (fig. 3 *A*). This striking picture seems to be evidence, par excellence, of some relation between phosphatase and tubular function. The atrophic cells are certainly capable of some degree of cellular metabolism and most assuredly contain many intracellular enzymes. Necrotic, dead or desquamated tubular cells [12] poisoned by nephrotoxic drugs still retain considerable phosphatase activity. It is evident that neither the death of the cell per se nor its actual physical disruption by drugs producing violent cellular damage is sufficient to inactivate or destroy the phosphatase, while a functional disturbance results in inactivity or disappearance of the enzyme. The cells whose phosphatase has completely disappeared are probably no longer functioning in their specific reabsorptive tubular activities.

Five of the rabbits subjected to complete ureteral obstruction were found at postmortem examination to have severe spontaneous interstitial nephritis in both the normal and the hydronephrotic kidney. This lesion is characterized by extensive interstitial cellular infiltration, most severe at the corticomedullary junction, with resultant blocking of the tubular system. The effect on the tubular segments proximal to the block is comparable to that following ligation of the ureter or more correctly ligation of the intrapelvic papilla. Grossly, these kidneys are finely pitted; the severity of the tubular injury may vary in different areas of the kidney, with early dilation and final atrophy. During this progressive change the phosphatase picture is comparable to that of the experimental hydronephrosis, with early fading and final loss of the enzyme.

The cat's glomeruli always reveal intense phosphatase activity, which does not disappear after ligation of the ureter.

Hydronephrosis in cats is somewhat different from that in rabbits, and in many ways the cat's kidney is unique. Unfortunately, the histochemical preparation of renal tissues from cats at varying periods of hydronephrosis gave somewhat erratic results. The stain was not at fault. Sometimes the phosphatase was present only in the glomeruli of the normal and the hydronephrotic kidney, or it was extremely faint in one and deep in the other. Histochemically, the phosphatase in the glomeruli and tubules of the cat behaves somewhat differently. It is exceedingly difficult to conjure any physiologic role of the enzyme in the glomeruli. The capillary endothelium in many tissues is extremely rich in phosphatase and perhaps the enzyme in the cat is related to the rich capillary glomerular tuft.

In spontaneous interstitial nephritis in rabbits the blood supply to the corresponding cortical segment is probably unaffected, yet phosphatase disappears. This disease disassociates the vas-

37. Ekehorn, G.: Acta med. Scandinav., 1931, supp. 36, p. 1.

cular and obstructive factors perhaps better than the experiments do.

A number of rabbits were subjected to varying periods of temporary occlusion of the left renal artery. The tubular lesions produced by this method are similar to those seen in the human

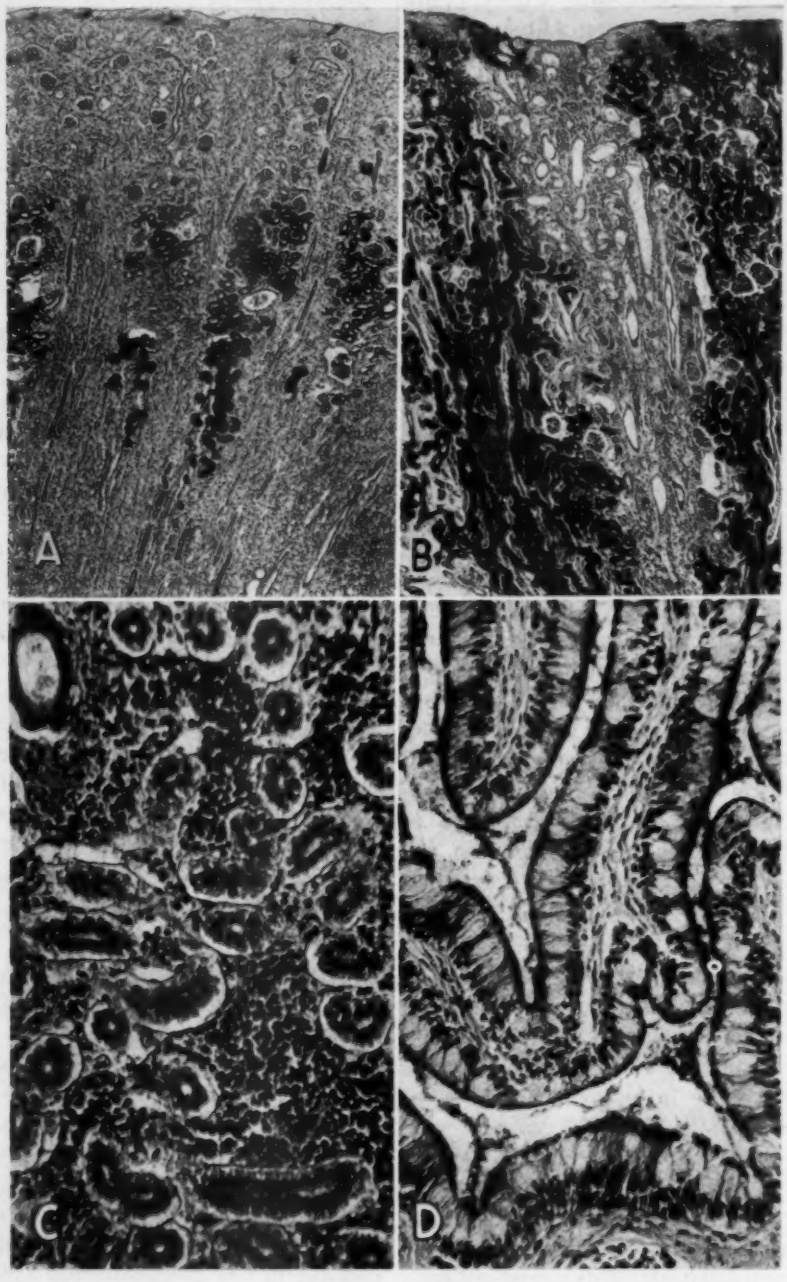

Fig. 3.—*A*, histochemical preparation for the demonstration of phosphatase activity in a rabbit kidney with two thirds of the intrapelvic papilla tied, and approximately one third of the collecting ducts unobstructed. Four or five nephrons still functioning in this section show normal dense phosphatase activity in their proximal convoluted tubules, while all other nephrons in the section are not functioning, and their tubules are atrophied, while the glomeruli are as yet unaffected. R-21; thirty-three days' obstruction; × 29.

B, histochemical preparation for the demonstration of phosphatase activity of the kidney not subjected to surgical interference in rabbit R-8. It reveals severe interstitial nephritis. Atrophic and dilated tubules are devoid of phosphatase, while neighboring tubules appear normal. In the lower left corner a small area of interstitial nephritis is seen. × 35.

C, similar preparation of the aglomerular kidney of a toadfish. The tubules are devoid of phosphatase, while interstitial lymphoid cells show moderate phosphatase activity. × 90.

D, similar preparation of the intestinal mucosa of the toadfish whose kidney is shown in *C*. The black band seeming to line the luminal portion of the epithelial cells indicates phosphatase activity. × 90.

"crush kidney" syndrome, according to Scarff and Keele.[38] The histochemical preparation of 5 of these kidneys showed a definite though small reduction in enzyme activity. Surprisingly, in an animal killed twenty-two hours after one hour of circulatory arrest the kidney was devoid of phosphatase. In a rabbit permitted to live for ten days following temporary circulatory arrest only a trace of phosphatase was present in the kidney, but extensive tubular calcification and degeneration were noted, so that the change could not be considered the primary result of an acute vascular episode. It is reasonable to believe that the alteration of the kidney would go on to primary atrophy.[39] The late lesions following such a procedure are essentially tubular.

Kinard and co-workers [17] showed that within hours following permanent ligation of the complete renal pedicle the phosphatase quantitatively and precipitously disappeared from the injured kidney. This experiment, of course, resulted in necrosis of the kidney with total and immediate loss of function. After the renal vein had been ligated for twenty-four or forty-eight hours I was able to demonstrate considerable phosphatase diffusely spread throughout cells which appear necrotic or nearly so. In 1 animal with unilateral renal ischemia and hypertension there was only moderate reduction in phosphatase activity.

Since the presumptive role of phosphatase in the proximal convoluted tubules is in the reabsorption of sugar, it was of great interest to determine the phosphatase activity of fish possessing aglomerular kidneys which can neither secrete nor excrete sugar.[40] Seven toadfish—marine teleosts, Opsanus tau—were studied and all of their tubules were devoid of phosphatase, although the interstitial pseudolymphoid cells apparently contained the enzyme. The advantage of the histochemical technic in this instance is obvious, for a biochemical determination would have further confused the problem by revealing the presence of phosphatase in extracts of the kidneys without revealing the fact that the tubules did not contain the enzyme. This observation should be confirmed in freshly caught aglomerular fish. If, perchance, the enzyme is demonstrated in the tubules of any of these fish which are unable to secrete sugars, it would make our conclusion untenable, namely, that the absence of phosphatase is specifically related to the inability of the renal tubules to transport sugars across their cells. If these tubules were able to secrete sugars, one would expect phosphatase to be on the vascular and not the luminal side of the cell since the transflux of the dextrose molecule would be in the opposite direction from that in the normal glomerular tubules. According to Smith,[41] the evolution of the aglomerular tubule is an adaptation to the oliguria associated with a sea water habitat, which medium is hypertonic to the blood of vertebrates. The aglomerular condition may then be considered as a terminal state in the progressive degeneration of glomerular function since there is no longer a need for elimination of large amounts of water. There is no reason to believe that the functional capacities of the aglomerular tubules whose only cells are cytologically proximal convoluted tubular epithelium are essentially different from those of the glomerular kidney. In accordance with sound principles of comparative physiology, there is continuity of function in the renal tubules as in other homologous organs.[41]

The intestinal mucosa of the toadfish shows the characteristic appearance of a "burned lumen" due to the phosphatase activity in the luminal border of the mucosal epithelium. This may be related to the absorption of sugars, which could subsequently be excreted by the gills. Unfortunately, the phosphatase activity of the gills was not studied, but its presence would not be essential since hexosephosphates might not have to be dephosphorylated before being excreted into the sea, as they apparently must be before being reabsorbed into the blood stream.

In a series of miscellaneous animals whose kidneys were prepared by the Gomori-Takamatsu technic, only the chicken revealed phosphatase in the glomeruli, and in this respect is now comparable to the cat. However, the glomerulus of the chicken has a fringe of capillaries around a central connective tissue core, and it is the connective tissue which reveals phosphatase activity. The significant findings in the other animals were the diffuse positive staining of the intertubular endothelium in a carp and a turtle and the failure to demonstrate phosphatase in the tubules of 1 salamander and 1 turtle obtained during November. Frogs are known to have phosphatase in their tubules. Starving frogs in winter may have no sugar in their blood,[42] in which case there would be no need for reabsorption of sugar in the tubules, but when sugar is injected into these frogs their tubules are immediately able to reabsorb it. The blood sugar of hibernating ground squirrels is only moderately reduced, according to Lyman. The significance of phosphatase in

38. Scarff, R. W., and Keele, C. A.: Brit. J. Exper. Path. **24**:147, 1943.

39. Maatz, R.: Franfurt. Ztschr. f. Path. **46**:438, 1934. McEnery, E. T.; Meyer, J., and Ivy, A. C.: J. Lab. & Clin. Med. **12**:349, 1927.

40. Marshall, E. K., Jr.: Physiol. Rev. **14**:133, 1934.

41. Smith, H. W.: The Physiology of the Kidney, New York, Oxford University Press, 1937.

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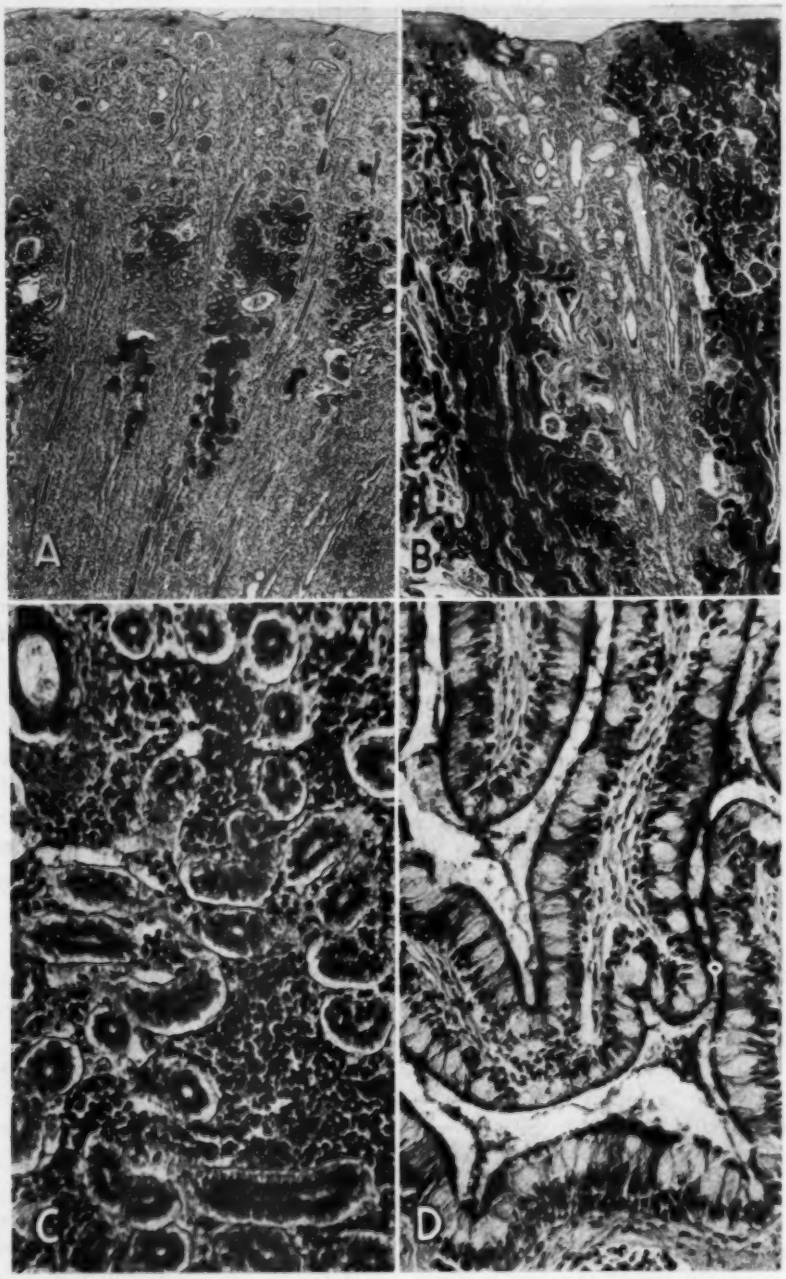

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the kidneys of amphibians and reptiles is open for interesting investigation.

A study of the oxygen consumption of the kidney which had not been surgically altered, but which, of course, was not strictly normal, and the hydronephrotic kidney showed that after six days or more of ureteral obstruction there was a significant difference in the respiration in an atmosphere of oxygen (fig. 4). With the exception of a kidney that had been hydronephrotic twenty-eight days in a pregnant rabbit the curve is remarkably smooth. Oxygen quotients of any tissue may vary considerably from one animal to another, apparently because of the metabolic state of the cells at the time of death. Inasmuch as only 1 rabbit was used for each interval, the smoothness of the curve may be fortuitous. But in any case the trend is obvious, and the important point is the consistently large difference in respiration after a short interval of hydronephrosis.

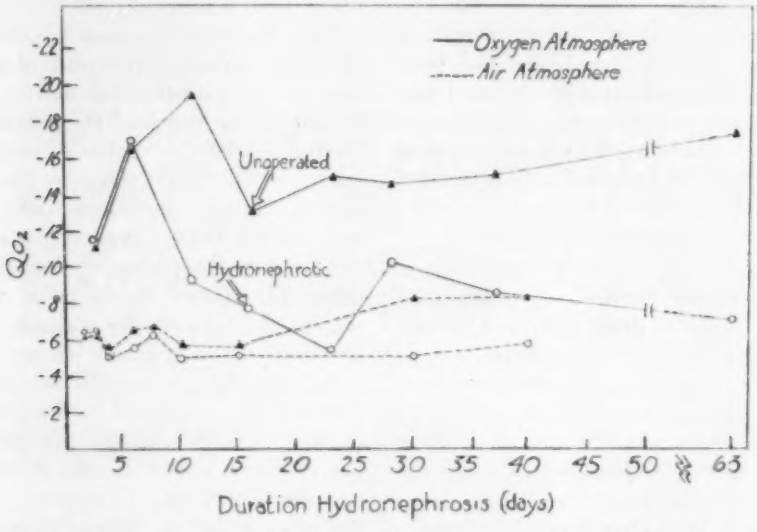

Fig. 4.—Graph showing oxygen consumption expressed as oxygen quotient (QO_2) of the hydronephrotic kidney and the kidney not subjected to operative interference at various intervals of hydronephrosis.

A comparable series of rabbits was studied under identical experimental conditions except that an atmosphere of air was used instead of oxygen. The maximum thickness at which tissue slices may be cut as given in the Warburg formula depends on satisfactory diffusion of oxygen into cells so that the central portion of the slice is not asphyxiated, and to satisfy this formula an atmosphere of oxygen must be used. It has been noted, however, that slightly greater than the calculated thickness gives satisfactory results.[43] In view of this observation and the conception that the hydronephrotic cortex may be in a state of ischemia and possibly reduced oxygen tension, it was decided to compare respiration in air and oxygen under similar experimental conditions. A slight difference between kidneys altered by operation and kidneys not altered in this manner was evident after several days but remained insignificant until fifteen days, after which time the right kidney, which had not been subjected to surgical interference manifested approximately the same consumption of oxygen as the hydronephrotic kidney in oxygen, while the oxygen consumption in the hydronephrotic kidney in air was still less. It is difficult to interpret these results in view of the differences in diffusion and oxygen tension, but in each experiment the right and left renal cortices are confronted with the same situation, and therefore each interval in itself is strictly comparable. Since the great bulk of the renal cortex consists of proximal convoluted tubules, it is inferred that the respiration is predominantly of those cells, and the difference might be in tune with their decreased function. Levy, Light and Blalock [44] have shown that there is a decrease in oxygen consumption and in blood flow in the Goldblatt kidney, and Raska [45] has demonstrated a low oxygen quotient for the ischemic kidney.

Hydronephrosis.—It is not altogether clear why phosphatase disappears from the hydronephrotic kidney. The simple explanation that it is a phenomenon of disuse, the tubules not being called on to reabsorb appreciable quantities of

43. Schorr, E., in A Symposium on Respiratory Enzymes, Madison, Wis., University of Wisconsin Press, 1942, p. 268.

44. Levy, S. E.; Light, R. A., and Blalock, A.: Am. J. Physiol. **122**:38, 1938.

45. Raska, S. B.: J. Exper. Med. **78**:75, 1943.

sugar, is unsatisfactory since phlorhizinized animals which do not reabsorb any sugar over periods of a week or more reveal no reduction in phosphatase activity. Phlorhizin does not inhibit the metabolism of renal tissue, while hydronephrosis does. Yet a purely metabolic explanation is not enough, for the ischemic kidney with reduced consumption of oxygen has rather intense phosphatase activity. The only occurrence to which I can attribute the loss of the enzyme is some specific tubular functional disturbance. It would be of interest to know whether phosphorylation proceeds at a decreased rate in the hydronephrotic cortex.

SUMMARY

By the use of the histochemical technic for the demonstration of phosphatase in the renal tubules it has been possible to correlate enzyme activity with tubular function.

Following complete or partial obstruction of a ureter of the rabbit or the cat, the phosphatase in the corresponding kidney disappears to a great extent or remains in small amounts.

Following temporary occlusion of the renal artery, ligation of the renal vein or permanent partial constriction of the left renal artery with resultant unilateral renal ischemia, phosphatase activity of the kidney is definitely though only slightly reduced.

Aglomerular toadfish kept in captivity do not have alkaline phosphatase in their renal tubules, and this is probably specifically related to the inability of this fish to secrete sugar.

Six days after a kidney has been rendered hydronephrotic by ureteral occlusion, the metabolism of tissue slices as measured by oxygen uptake in the Warburg apparatus reveals a significant difference between the hydronephrotic kidney and the kidney not subjected to surgical interference.

Phosphatase probably plays a role in the reabsorption of sugar by dephosphorylating hexosephosphates. The relative decrease or almost complete disappearance of the enzyme when tubular function is disturbed, as in hydronephrosis, spontaneous interstitial nephritis or ligation of the intrapelvic renal papilla, is probably a sensitive reflection of the abatement or reduction in glomerular filtration and tubular reabsorptive activity.

PANCREATIC FUNCTION AND DISEASE IN EARLY LIFE

V. PATHOLOGIC CHANGES ASSOCIATED WITH PANCREATIC INSUFFICIENCY IN EARLY LIFE

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BOSTON

It has been shown that an obstructive lesion of the pancreas, characterized by inspissation of secretions in acini and ducts, dilatation of ducts, atrophy of parenchyma and variable degrees of fibrosis, is found in constant association with at least two quite dissimilar clinical pictures in infancy and childhood: (1) meconium ileus in the neonatal period and (2) a severe nutritional disturbance, recognizable clinically after the first few weeks of age and characterized frequently by many of the features of the celiac syndrome, particularly after the patient has reached the age of 1 year.[1] Respiratory disease usually is present with the nutritional disturbance and often is responsible for what was until recently a uniformly fatal outcome. The symptoms referable to the respiratory tract may dominate the clinical picture and even obscure the nutritional aspects of the disease. Pancreatic hypochylia or achylia, which may be anticipated from the obstructive nature of the lesion in the pancreas, has been demonstrated in neonates with meconium ileus and in infants and children with the nutritional and respiratory forms of the disease.[2] The explanation for such divergent clinical pictures caused by the same lesion is found in the time of occurrence of the pancreatic achylia: If the achylia is present during the latter part of intrauterine life, a physically altered meconium will result which will cause intestinal obstruction shortly after birth; if the achylia is not present until after the neonatal period, the disease picture will be that of the celiac syndrome. It is my purpose in this paper to describe some of the important pathologic changes in patients with pancreatic insufficiency on the basis of 87 postmortem examinations performed in the department of pathology of the Children's Hospital.

A general discussion of pancreatic insufficiency in early life has been given elsewhere.[1b]

PRESENT STUDIES

Findings common to the group were emaciation, absence of fat in the usual depots of the body, atrophy particularly of the musculature in the buttocks, loose skin, delayed skeletal development, varying degrees of rickets, scurvy and vitamin A deficiency. They will not be considered in greater detail except as noted in subsequent pages. Attention will be directed particularly to the pancreas, the respiratory system, the liver and the salivary glands.

Pancreas.—The pancreas varied greatly in gross appearance. It usually was firmer, thinner and somewhat smaller than normal. In some instances no gross abnormalities could be detected, and in others the external surface was distinctly irregular because of the great variation in size and shape of the lobules of pancreatic tissue and the shrinkage of the fibrosed portions at intervals below the original level of the surface of the gland (fig. 1). Gross dissection of the pancreatic ducts frequently was not possible because of the small size of those structures in infants. In some instances definite narrowing was found. Malformation of the pancreatic ducts associated with an annular type of pancreas was an occasional finding. In the majority of specimens examined, however, congenital atresia or stenosis was not present. On the contrary, the pancreatic ducts more often were dilated, particularly in the head of the gland, and on cross section small yellow to white plugs in the dilated ducts could be identified sometimes with the naked eye. Great variation existed, too, in the histologic picture (fig. 2). The most common finding was a striking degree of atrophy of the exocrine parenchyma of the organ, with an increase in the amount of connective tissue, which often was more apparent than real, since coalescence of the fibrous stroma of the gland, following loss of acinous tissue, accentuated the diffuse replacement fibrosis. Small and large

From the departments of pathology of Harvard Medical School, the Infants' Hospital and the Children's Hospital.

These studies were supported in part by the Ann Proctor Rice Fund of Harvard Medical School and in part by a grant to the Children's Hospital from the Lederle Laboratories.

1. (*a*) Andersen, D. H.: Am. J. Dis. Child. **56**:344, 1938. (*b*) Farber, S.: New England J. Med. **229**:653 and 682, 1943.

2. (*a*) Farber, S.; Shwachman, H., and Maddock, C. L.: J. Clin. Investigation **22**:827, 1943. (*b*) Andersen, D. H.: Am. J. Dis. Child. **64**:643, 1942.

ducts usually were dilated and filled with a homogeneous eosinophilic material, which frequently was laid down in concentric formation. The small ducts, and particularly the acini, were filled with this homogeneous eosinophilic material, which had the histologic appearance of inspissated secretion. Large mononuclear and lymphocytic infiltration was noted in some instances, but neither necrosis nor acute inflammatory changes were encountered. The islands of Langerhans did not partake actively in the pathologic process.

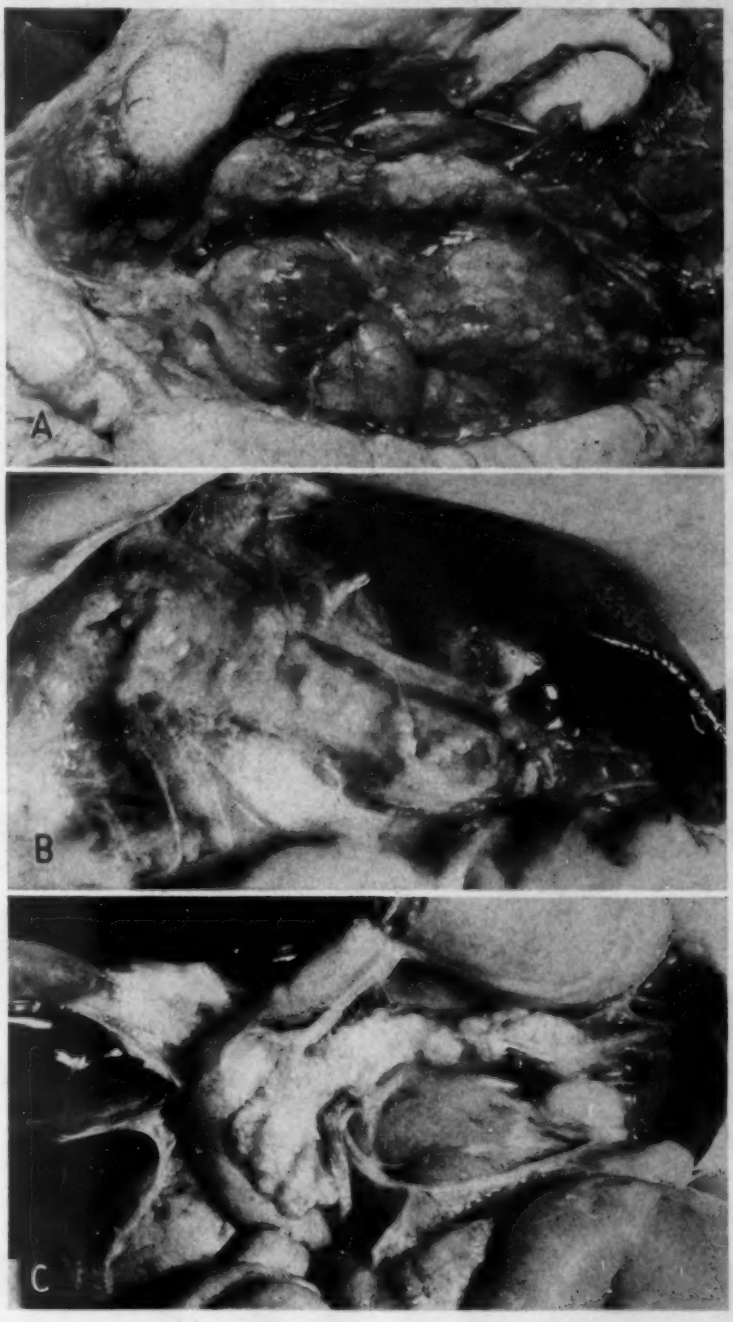

Fig. 1.—Three examples of pancreatic fibrosis. Note the irregularity and distortion of surface, which was caused by contraction of connective tissue leaving abnormally shaped islands of pancreatic tissue projecting above the surface. *A* shows the pancreas of a child 5 7/12 years of age; *B*, that of a child 3 10/12 years of age, and *C* that of an infant 3 weeks of age with meconium ileus.

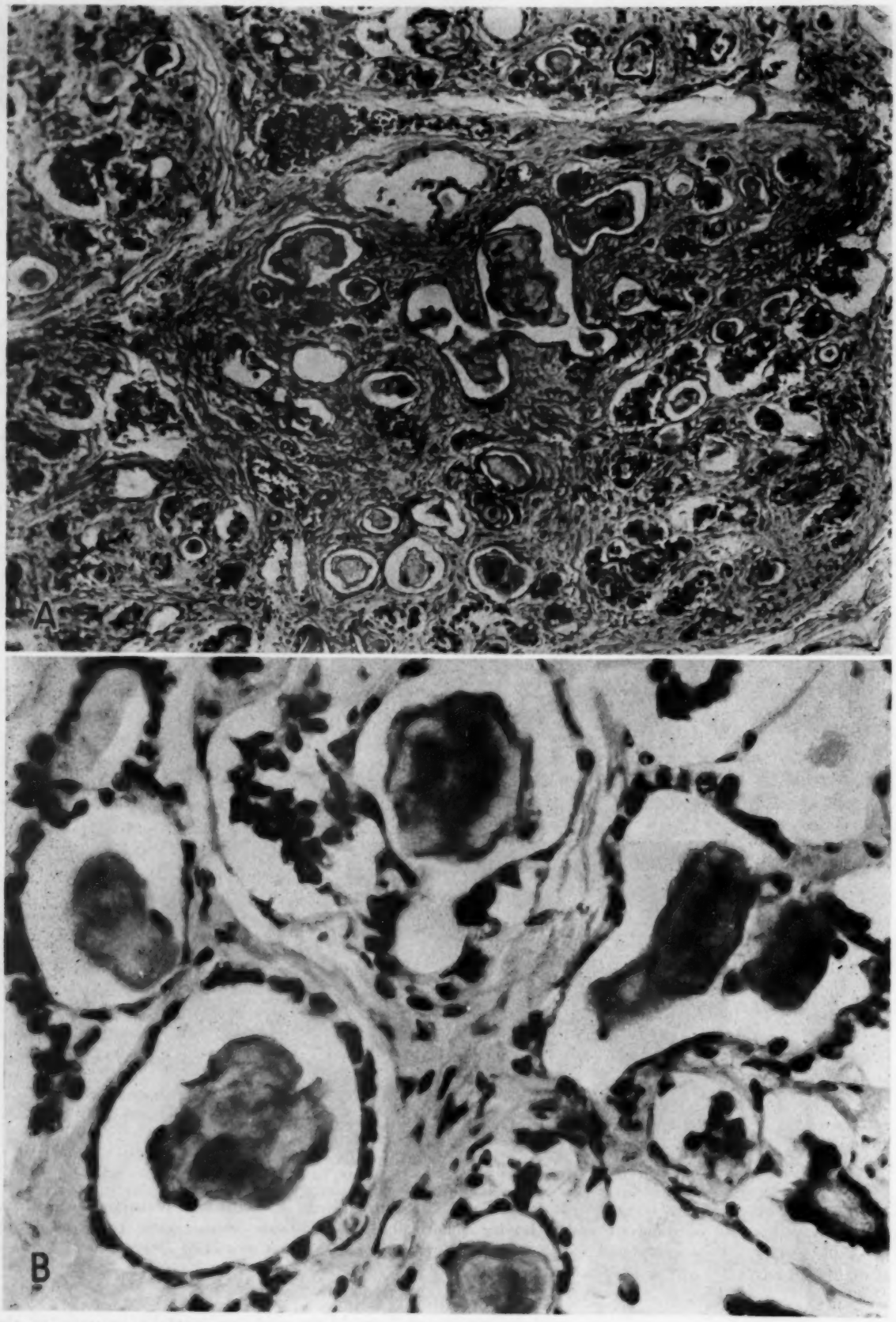

Figure 2

(See legend on opposite page)

Two extremes of the histologic picture were observed: What appeared to be the end result of the process was noted in the almost complete disappearance of the acinous tissue. The bulk of the gland was composed of connective tissue interspersed with dilated ducts filled with inspissated material and collections of islets, which had coalesced because of the great loss of intervening acinous tissue. The other extreme was represented by what appears to be the early stage of the process. Here no increase in fibrous tissue, apparent or real, was noted, and the ducts were still within normal limits in diameter. The lumens of the small ducts, however, and the acini contained homogeneous eosinophilic masses of inspissated material. The picture here was interpreted as indicative of widespread internal obstruction to the outflow of pancreatic juice.

Except for those instances in which there was congenital obstruction in the main pancreatic ducts, the process appeared to be one of internal obstruction to the outflow of the products of the exocrine portion of the pancreas, as proposed by Wolbach, with the obstruction beginning in the individual cells of the acini. This is followed in time by atrophy of acinous structures, fibrosis of the gland and dilatation of the ducts, giving a picture equivalent to that produced by ligation of the pancreatic ducts. The functional consequence of such intrinsic obstruction is pancreatic hypochylia or achylia, which is present from the time inspissation occurs in a sufficient number of acinous structures in the pancreas and long before there are produced the atrophy of these structures, the fibrosis of the organ and the dilatation of ducts which are the later consequences of intrinsic obstruction. This was demonstrated in an infant of 14 months who exhibited many of the characteristic clinical features of the celiac syndrome. No evidence of pancreatic enzyme activity was found on analysis of the duodenal content. Soon thereafter, at postmortem examination the pancreas appeared normal on gross examination, and no evidence of atrophy of acinous structures, fibrosis of the organ or dilatation of the larger ducts was found on histologic study. The small ducts and the acini contained inspissated secretions, however, which appeared to obstruct effectively the outflow of pancreatic juice (fig. 3). That the clinical picture of the pancreatic form of the celiac syndrome and pancreatic achylia may be produced by a lesion of the pancreas which involves only the acini and small ducts suggests strongly that the importance of these widespread early lesions has escaped recognition in similar instances where correlation with determinations of pancreatic enzyme activity was not possible.

The reversibility of this process in the pancreas, should the cause of the disturbance be cured or arrested, is a matter of great importance. Little is known of the regenerative powers of the human pancreas. Mention of such a possibility was made by Hess and Saphir.[3] My associates and I have recently had several opportunities to gather data bearing on this question. A boy 12 months of age at the time of death suffered from convulsive episodes which study showed were associated with hypoglycemia. A three stage pancreatectomy was performed without the finding of an islet adenoma. At the third operation, all visible remaining portions of the pancreas were removed, and the common duct was anastomosed to the duodenum. About eight months after the last operation, the patient died. At autopsy a piece of pancreas measuring 2 by 1.5 by 1.2 cm. was found in the usual location of the pancreas. The amount of pancreas present appeared to be more than one-half the normal amount for the age (fig. 4). My associates and I [2a] have described elsewhere the results of studies on pancreatic enzymes in the duodenal contents of patients with hypoglycemia from whom portions of the pancreas were removed at operation. One patient from whom approximately 80 per cent of the pancreas had been operatively removed showed five weeks after the operation somewhat less than half the expected normal tryptic activity for the age but more than enough for the needs of the body. Lipolytic activity was decreased also. Amylolytic activity was definitely elevated. It appears clear that the pancreas has a strong power of regeneration in the infant or the child and that comparatively little functioning pancreatic tissue may meet those needs of the body served by pancreatic enzymes.

Respiratory Tract.—The lungs of those patients who lived for more than a few months after birth were almost invariably diseased. Hyperexpansion of the lungs, most prominent an-

3 Hess, J. H., and Saphir, O.: J. Pediat. **6**:1, 1935.

EXPLANATION OF FIGURE 2

Fig. 2.—*A*, photomicrograph of a section of a pancreas showing dilatation of ducts containing dense inspissated material. Note disappearance of acini and increase in connective tissue. Islets are present in large numbers because of loss of exocrine parenchyma.

B, high power detail of dilated small ducts of the pancreas seen in *A*. Note dense eosinophilic material in the lumens of the ducts.

teriorly at the time of autopsy, was the usual finding (fig. 5*A*), and this was the result of the partial obstruction in the trachea, the bronchi and the bronchioles caused by the thick tenacious mucoid and mucopurulent exudate (fig. 5*B*). At times the impression was gained that the patient finally must have experienced suffocation because of the abundance and tenacity of the mucopurulent exudate in the upper respiratory tract (fig. 6). The process in the lungs was characterized usually by bronchiectatic and bronchiolectatic abscesses, thickening of bronchiolar walls and varying amounts of bronchopneumonia. Either emphysema or obstructive atelectasis was noted, depending on the presence of complete or incomplete obstruction in the bronchial and bronchiolar supply to different parts of the lung. Where the process was one of long duration, considerable fibrosis of the lung was found, and a chronic interstitial form of bronchopneumonia was superimposed on the original process (fig. 7). In most instances the causative agent was Staphylococcus aureus, which appeared usually to be of rather low virulence, as shown by its lack of invasiveness and by the relatively small amount of damage to the lung even after an infection of many months' duration.

A prominent finding was the distention of the mucous glands of the trachea and bronchi, which were filled with a thick inspissated material similar in histologic appearance to that found in the pancreatic acini and ducts. A similar condition was present frequently in the sublingual, submaxillary and parotid glands and in the mucous glands of the esophagus, the duodenum, the gallbladder and the jejunum and even in accessory pancreatic tissue in the wall of the stomach and the wall of the small intestine (fig. 8).

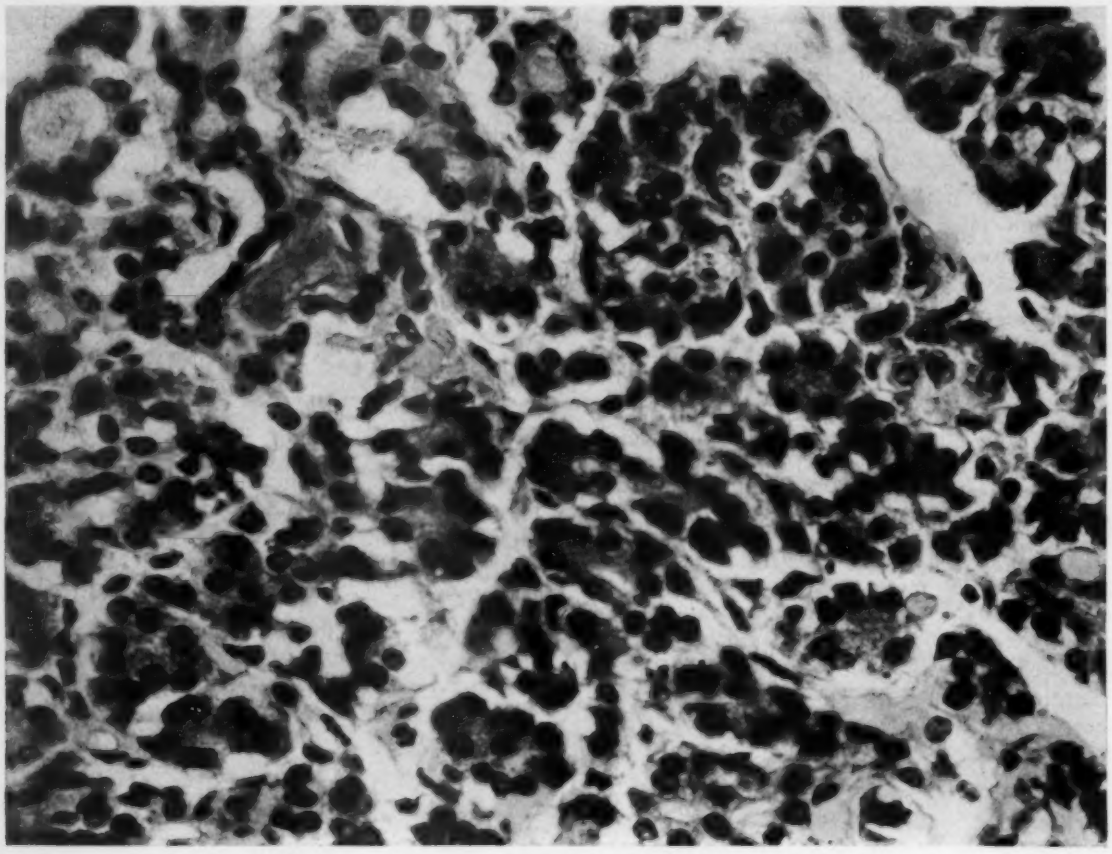

Fig. 3.—Photomicrograph of a section of the pancreas of a patient 15 months of age. Absence of pancreatic enzyme activity in the duodenal content was demonstrated. There is filling of small ducts and acini with dense eosinophilic inspissated material. Note absence of fibrosis, atrophy and dilatation of ducts. Hematoxylin and eosin.

Liver.—The gallbladder often was small. The bile was thicker and more sticky than normal. The liver usually was increased in size, had a yellowish tongue and was somewhat softer than is normal. The hepatic cells contained large amounts of fat, which was present in large droplets in the cytoplasm. This was stained easily with sudan IV and was diffusely scattered throughout the organ. In the few cases in which

no fat metamorphosis of the liver was noted, the pancreas showed only small amounts of atrophy. A rough correlation could be made between the amount of fat in the liver and the degree of atrophy and fibrosis of the pancreas. This recalls the observation of Cole and Howe,[4] who described what they called "the pancreaticohepatic syndrome" in adults, characterized by a severe grade of fatty infiltration of the liver and fibrosis of the pancreas. Etiologic factors of importance in their patients were pancreatic lesions or disease of the gallbladder. In consideration of the experimental work of Dragstedt,[5] Cole and Howe [4] suggested that the fatty condition of the liver was secondary to the pancreatic lesion. In their adult patients the islets appeared normal histologically as they do in patients with pancreatic fibrosis in early life.

a size many times the normal, were filled with a thick inspissated eosinophilic material resembling that found in the pancreatic ducts and acini of the same patients (fig. 10). This material contained no bile pigment and consisted probably of mucoproteins elaborated by the lining cells of the greatly dilated bile ducts. The sequences leading to these obstructive changes appear to be similar to those in the pancreas. The inspissation of the mucoproteins in some but not all of the finer intrahepatic bile ducts leads to obstruction and dilatation of ducts. Finally atrophy of the parenchyma draining into these ducts is followed by replacement fibrosis and condensation of the stroma, which produce cirrhosis and distortion of hepatic structure. The microscopic picture in the liver duplicates in all important findings the lesions in the pancreas, even to the

Fig. 4.—Region of the pancreas and the duodenum showing a portion of pancreas estimated to represent more than one-half the normal amount for the age, which had regenerated apparently in the eight months since an attempted total removal of the pancreas from a boy 12 months of age.

The lipid partition of two livers from patients with pancreatic fibrosis in our series was studied by S. J. Thannhauser, who has published the detailed analyses.[6] In one the lipids were found to be essentially normal. In the other there was a great increase in the neutral fat fraction, and the cholesterol and the phospholipids were relatively reduced.

In a small number of patients with the pancreatic form of the celiac syndrome there was found an unfamiliar type of cirrhosis of the liver (fig. 9). Icterus was never observed in these patients. The cirrhosis appeared to be secondary to intrahepatic biliary obstruction. In these cases dilated small bile ducts, which reached

color and the appearance of the inspissated secretions (fig. 10).

COMMENT

Four general theories have been advanced to explain the pancreatic lesions and certain of the other pathologic changes:

1. Congenital malformations of the pancreatic ducts have been found in a small number of instances, particularly in those patients with meconium ileus who died in the neonatal period. Excellent pathologic studies in such instances have been published by Kornblith and Otani [7] and by Hurwitt and Arnheim.[8] The suggestion has been made that there is a general tendency

4. Cole, W. H., and Howe, J. S.: Surgery **8**:19, 1940.
5. Dragstedt, L. R.: J. A. M. A. **114**:29, 1940.
6. Thannhauser, S. J., and Reinstein, H.: Arch. Path. **33**:646, 1942.
7. Kornblith, B. A., and Otani, S.: Am. J. Path. **5**:249, 1929.
8. Hurwitt, E. S., and Arnheim, E. E.: Am. J. Dis. Child. **64**:443, 1942.

toward atresia of ducts since atresia or stenosis of epithelium-lined passages has been an occasional additional abnormality. To explain both the respiratory and the intestinal symptoms of the disease, Rauch and his co-workers offered an ingenious theory based on the fact that the anlages of the lungs and the pancreas are derived from a common endodermal tube in close prox-

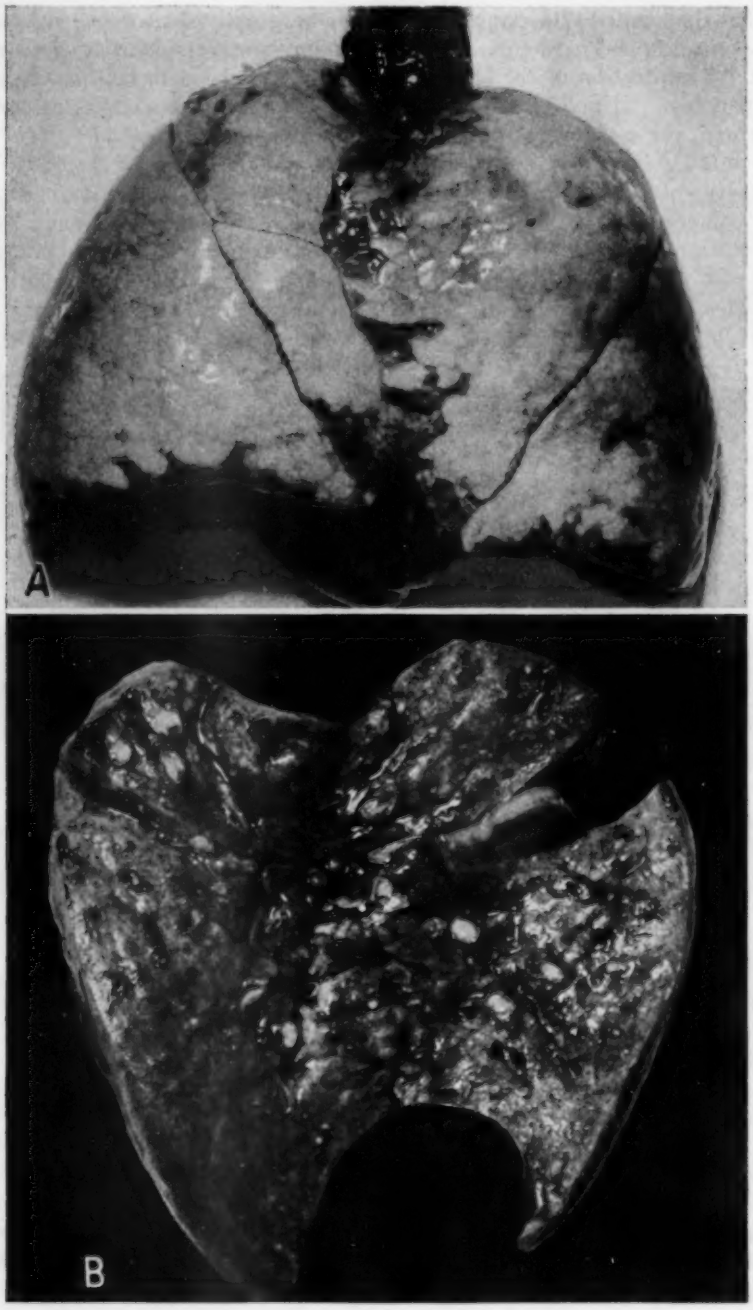

Fig. 5.—*A*, the lungs of a patient aged 5 7/12 years with the celiac syndrome. Pancreatic fibrosis was demonstrated at autopsy (fig. 1 *A*). Note the marked hyperexpansion with focal areas of atelectasis, particularly along the margins of the lungs.

B, a cut surface of a lung shown in *A*. The bronchi and bronchioles are filled with thick mucopurulent material, a finding which accounts for the gross appearance of the external surface of the lung.

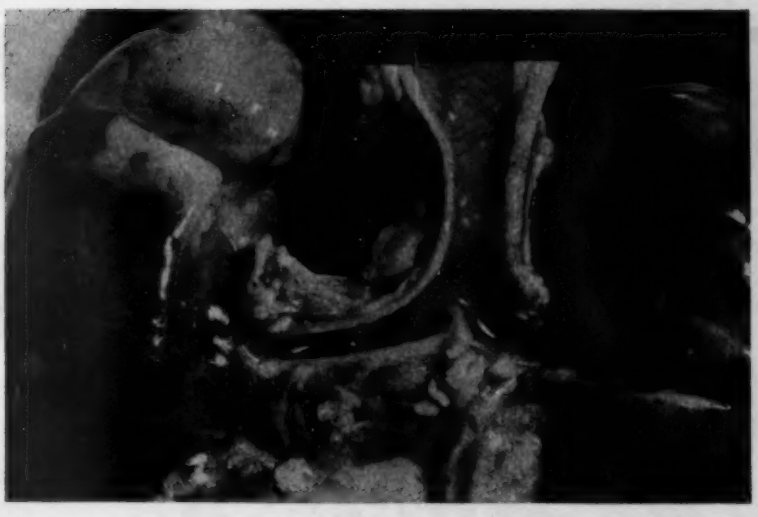

Fig. 6.—Posterior view of the opened trachea and the major bronchi of an infant aged 20 months with pancreatic achylia and pancreatic fibrosis. The trachea and bronchi are filled with thick, tenacious mucus and purulent material, which is removed with difficulty.

Fig. 7.—Cut surface of a fixed lung from a patient aged 10 months with pancreatic achylia and pancreatic fibrosis. Note overexpansion and small scattered areas of consolidation, many of which represent abscesses. Large areas of the lung are uninvolved.

inity to each other.[9] No evidence has been produced, however, to show that such an explanation holds for most patients with pancreatic fibrosis, and indeed in the majority of instances of pancreatic fibrosis no evidence of congenital malformation of ducts can be found nor are congenital malformations demonstrable elsewhere in the body. An important piece of evidence which points to the conclusion that congenital obstruction of the pancreatic ducts does not occur in most patients with pancreatic fibrosis is found in the fact that a history of meconium

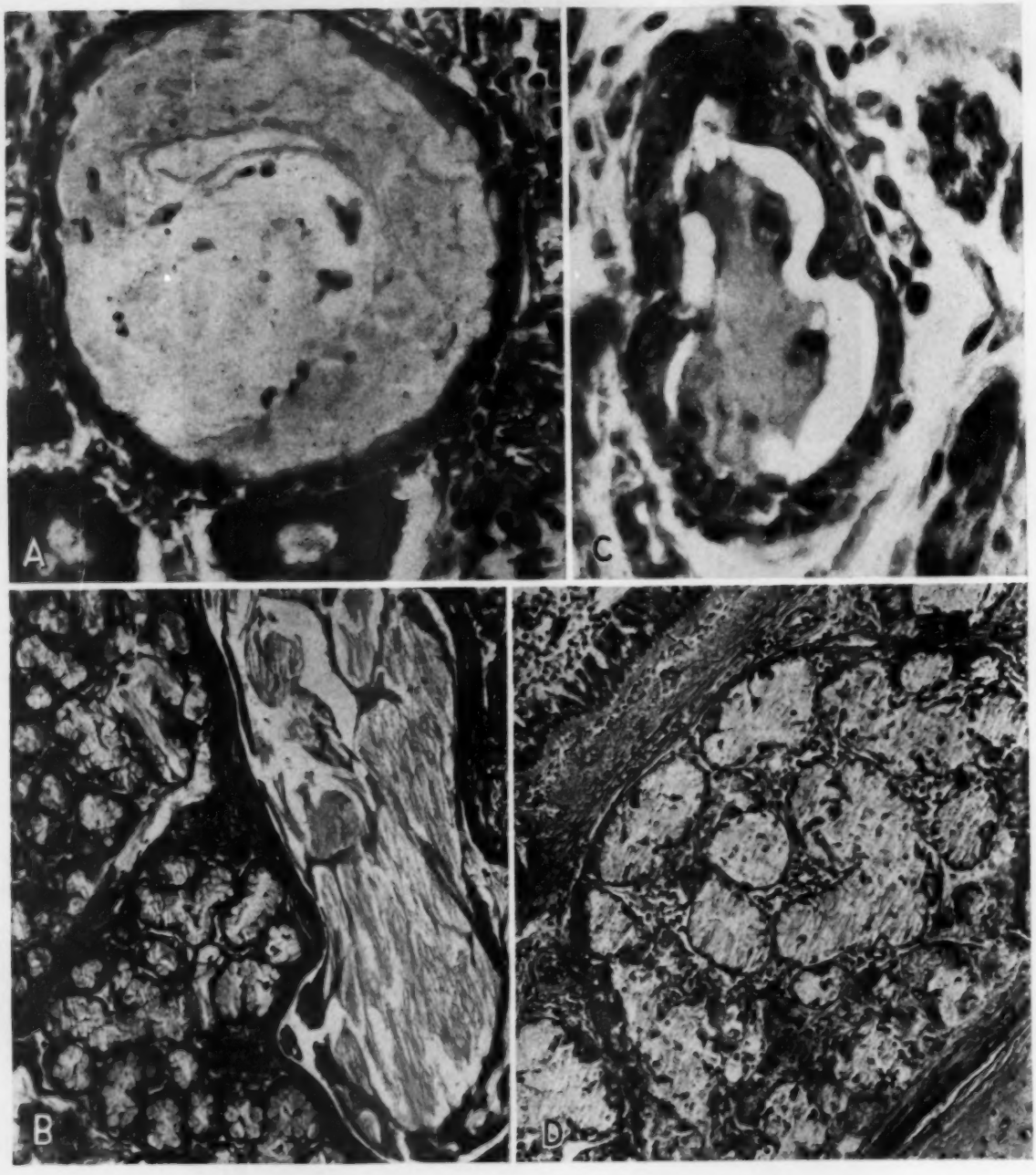

Fig. 8.—High power photomicrographs of sections from organs of a patient with pancreatic achylia and pancreatic fibrosis; hematoxylin and eosin. *A*, dilated gland in the duodenum filled with inspissated mucus. *B*, sublingual gland. Note inspissated mucus in the large duct and in the acinous cells. *C*, dilated gland in the esophagus containing inspissated secretion. *D*, wall of trachea. Note submucosal glands distended and filled with thick mucus.

9. Rauch, S.; Litvak, A. M., and Steiner, M.: J. Pediat. **14**:462, 1939.

ileus is not given for those patients who survive the neonatal period and who have symptoms of a nutritional disturbance that begins some time after meconium has been emptied from the bowel. If congenital obstruction of ducts were the cause of the pancreatic fibrosis, meconium ileus should be a constant finding in all patients with this lesion, and such is not the case.

2. Considerable attention has been called to the role of vitamin A deficiency in the consideration of the pancreatic lesions and of the pulmonary changes on the basis of pathologic evidence of vitamin A deficiency and of malabsorption of vitamin A.[18] No evidence has been produced so far to show that congenital deficiency of vitamin A is the cause of either the pancreatic or the pulmonary changes. It is possible for sufficient anatomic change referable to vitamin A deficiency, such as keratinizing metaplasia of the duct epithelium in the pancreas and of tracheal and bronchial epithelium, with subsequent obstruction of the pancreatic ducts and of the respiratory passages by masses of keratinized debris, to be present to account for real obstruction, but this is a rare event.[10] Despite the evidence of greatly diminished absorption of vitamin A, important lesions caused by deficiency of vitamin A are not seen commonly at autopsies on these patients. They are found so irregularly and when present comprise such a small part of the pathologic picture that the conclusion seems warranted that vitamin A deficiency is not the cause but is rather a complication of the pancreatic disease. As such, it explains certain of the symptoms and disturbances of function in the gastrointestinal and pulmonary systems noted in this disease.

Fig. 9.—A markedly cirrhotic liver from a patient aged 7 months who had pancreatic achylia.

3. The frequent occurrence of chronic pneumonia in infants with pancreatic fibrosis and the finding of inclusion bodies in the epithelial cells of the pancreas and the upper respiratory tract or the salivary glands in certain instances have raised the question of the role of a filtrable virus as the etiologic factor in this condition. In the first example of vitamin A deficiency in man recorded, by Wilson and Dubois,[11] inclusion bodies in the salivary glands, pancreatic fibrosis and vitamin A deficiency were present in the same patient. Brody[12] recently encountered inclusion bodies in 2 of 3 cases of pancreatic fibrosis and was able to find 5 more observations of this type in the literature. A review of 50 postmortem examinations on infants and children with pancreatic fibrosis carried out with Dr. Edward Pratt in our laboratory disclosed the presence of intranuclear and cytoplasmic inclusions in the salivary glands, the lungs, the trachea or the pancreas in 12 per cent of the series, an incidence which is not greater than that of similar inclusion bodies in the autopsy population in this age group in the same laboratory.[13] The possibility of a virus disease superimposed on the initial disease process cannot be excluded. No evidence has been provided in support of the suggestion that a virus infection is of primary importance.

4. The prominence of the inspissated secretion of the pancreas in this disease suggested to Wolbach more than twenty years ago that the genesis of this striking lesion could be attributed to the production of an abnormal pancreatic secretion. This he stated in a pathologic section of a paper on vitamin A deficiency, the clinical portion of which was written by Blackfan,[10] as follows: "Our preliminary studies indicate that the pathogenesis of this striking pancreatic affection resides in the production of an abnormal secretion which inspissates and leads to disten-

10. Wolbach, S. B., cited by Blackfan, K. D., and Wolbach, S. B.: J. Pediat. **3**:679, 1933.

11. Wilson, J. R., and DuBois, R. O.: Am. J. Dis. Child. **26**:431, 1923.

12. Brody, H.: New York State J. Med. **31**:1256, 1941.

13. Farber, S., and Wolbach, S. B.: Am. J. Path. **8**:123, 1932.

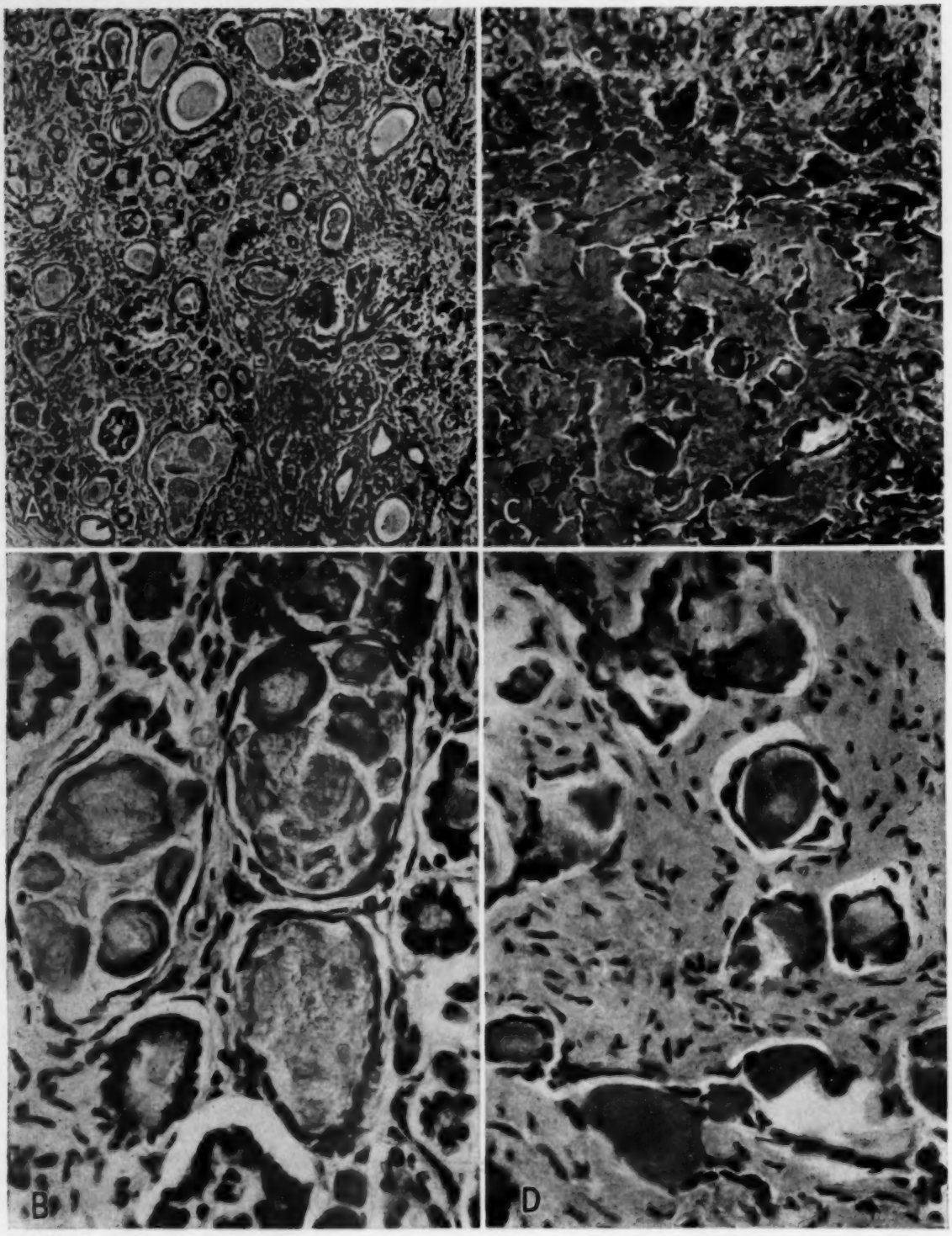

Fig. 10.—Photomicrographs of sections from the pancreas and the liver of a 7 month old infant with pancreatic achylia; hematoxylin and eosin. *A*, low power magnification of pancreas. Note dilated, secretion-filled ducts, diffuse fibrosis, atrophy of acini and the large number of islets in the field. *B*, high power detail of pancreas showing masses of eosinophilic inspissated secretions in dilated ducts lined by flattened epithelium. *C*, low power magnification of liver showing dense fibrous tissue and numerous dilated small bile ducts filled with eosinophilic inspissated material. Note the remnant of recognizable liver tissue at the top. *D*, high power detail of the same liver. Note similarity to pancreas.

sion and atrophy of ducts and acini." Subsequent postmortem studies have given support to this suggestion. The obstructive process begins, then, in the acini themselves. Interference with the liberation or with the proper formation of pancreatic enzymes is the process of fundamental importance. Secondary obstruction of the acini and small ducts and later of the large ducts by a physically altered pancreatic secretion then takes place, to be followed in turn by atrophy of the acini and replacement fibrosis of the organ. Dilatation of ducts giving an appearance which has been described as "cystic" is a consequence of the obstruction by the altered secretions.

The finding of inspissated materials in the mucous glands of the trachea, bronchi, esophagus, duodenum, gallbladder and salivary glands and even in accessory pancreatic tissue gives evidence that the pancreas is only one of many structures of the body involved in a process the basic feature of which is the physical alteration of secretion, which inspissates, obstructs and causes dilatation of acinous or mucous structures in various parts of the body. An explanation for the pertussis-like cough and the asthma-like respiratory complaints may be found in the thick mucus which is produced in the trachea and bronchi and which is expelled with difficulty. The accumulation of thick mucus causes respiratory obstruction. The failure of lubrication of ciliated epithelium in the upper respiratory tract due to the lack of normal thin mucus permits secondary infection with Staph. aureus of relatively low virulence. The respiratory signs therefore depend on primary obstruction by thick mucus, failure of proper lubrication of ciliated epithelium and secondary staphylococcic infection.

Congenital stenosis or atresia of the pancreatic ducts or malformations of the duct system associated with an annular type of pancreas are rare causes of pancreatic achylia. Should the malformation of the pancreatic duct system be the only congenital defect in the body, the disease produced by the pancreatic achylia is not systemic in distribution. In such patients the clinical problem is limited to pancreatic substitution therapy without the serious complication of pulmonary disease. A discussion of these considerations with particular reference to the prognosis for patients with meconium ileus has been given elsewhere.[14] Except in those patients in whom a congenital malformation limited to the pancreatic ducts is found the cause of achylia, the disease is not to be regarded as one involving only the pancreas, although in the majority of the patients it is the diseased pancreas which is responsible for most of the gastrointestinal symptoms. The material in the pancreas and in the various glands involved suggests the thick mucilaginous material in the upper respiratory tract of patients with asthma. That an element of spasm in the ducts of the pancreas and other glands involved may play a role in this disease is a possibility which appears to be worthy of further study.

If the suggested conception of the nature of the alteration in the pancreas is correct, the process must be regarded as a reversible one as long as there is present some viable exocrine parenchyma from which regeneration may take place. The evidence of the systemic nature of the disease produced by postmortem studies and a reevaluation of clinical findings call for less emphasis on the symptoms of the nutritional disturbance, which so long obscured the true nature of the disease, because of the close resemblance to the clinical picture of idiopathic celiac disease, and point to the possible occurrence in some patients of disease of the lungs, the upper respiratory tract, the liver and the intestinal tract without involvement of the pancreas. The validity of this suggestion has already been proved by the finding of alterations in the lung and in the liver of the type described in this paper in a patient who showed no clinical symptoms referable to pancreatic achylia and in whom at autopsy a norml pancreas was found. Such observations mark the final evidence necessary for a complete separation, etiologic, pathologic and clinical, between the condition termed idiopathic celiac disease and the condition which for so long was considered identical with it and which is now termed, for want of a better name, pancreatic fibrosis. In idiopathic celiac disease there is no known pathologic picture and the disease does not appear to be a systemic one. In what is called, for convenience, pancreatic fibrosis there are definite pathologic changes in the pancreas, and the pancreas shares these changes with many other structures in the body.

The widespread involvement of mucus-secreting structures in the body as shown by the change in the physical character of the mucus produced suggests the possible importance of either a deficiency or an insufficiency of the mucinase required for the maintenance of mucus in a normal physical state. The character of

14. Farber, S.: Pancreatic Function and Disease in Early Life: IV. The Relation of Pancreatic Achylia to Meconium Ileus, J. Pediat., to be published.

the duodenal drainage and the appearance of the inspissated secretions in the acini and small ducts are reminiscent of the consequences of vagal stimulation. A disturbance of parasympathetic innervation or, perhaps more accurately, an autonomic imbalance in the nervous control of secretion in the pancreas and in mucous glands is suggested by these findings.

The studies reported here indicate that an investigation, both clinical and experimental, of the nervous control of secretion in the pancreas and other glands of the body represents a logical and promising approach to an understanding of the nature of this systemic disease.

SUMMARY

In 87 infants and children with pancreatic insufficiency postmortem examination revealed changes in the lungs, the upper respiratory tract, the liver, the gallbladder and the upper alimentary tract which were essentially the same as those in the pancreas. They appeared to depend on a primary alteration in the character of secretions within glandular structures, leading to obstruction and loss of function. These studies indicate that what has been called cystic fibrosis of the pancreas or pancreatic fibrosis is really a systemic disease with a variety of clinical appearances, the occurrence of which depends on when the obstructive changes occur and which organs are affected. The direction of further research into the nature of this systemic disease is indicated by these studies. An investigation of the nervous control of the secretion of the pancreatic and of the mucous glands appears to be a logical approach to the nature of this systemic disease, of which pancreatic achylia is so characteristically a part.

ANALYSIS OF THE RELATIONSHIP OF SEMINOMA AND ARRHENOBLASTOMA TO TERATOMA

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The origin of embryonal carcinoma or seminoma of the testis and that of arrhenoblastoma of the ovary have been the subject of prolonged debate. Inclusion of unrelated tissues has occasionally been observed in these tumors; in rare instances all germinal layers were represented. One school of thought believes that these tumors are forms of teratoma in which one element has a marked tendency to overgrow the other structures. The opposing group finds the evidence in favor of this concept too meager to be convincing, since numerous apparently homogeneous tumors are encountered, while in others the admixtures appear insignificant.

Ewing [1] sought to demonstrate the teratomatous origin of seminoma, and his views have found wide acceptance. He was "forced to conclude that this common tumor of the testis is always a one-sided development of a teratoma . . . yet Chevassu, Debarnardi, and others derive this tumor from the spermatoblasts or their embryonal equivalents, and separate this group from teratomas with which they are so often associated."[2]

Several investigators have suggested the teratomatous origin of arrhenoblastoma (Krock, 1933; McLester, 1936; Kauter and Klawans, 1940). Krock and Wolferman [3] analyzed the 70 cases available in the literature and found suggestions of tridermal tissue elements in 24. They were reluctant to recognize arrhenoblastoma as a pathologic entity and recommended that it be classified as a form of teratoma with one-sided development.

In his discussion of Krock and Wolferman's paper, Novak [4] said that the evidence in favor of a teratomatous origin of arrhenoblastoma was not convincing. Meyer's [5] concept of the origin of arrhenoblastoma is still widely shared. He suggested that arrhenoblastoma originated from male-directed cells which had persisted from an early stage of gonadal development.

One may conclude from a review of the literature that the teratomatous origin of seminoma has been widely accepted on Ewing's authority. On the other hand, Meyer has won a large following for the nonteratomatous nature of arrhenoblastoma. However, the pertinent facts in both instances are similar. In the majority of cases both these tumors appear homogeneous; in some cases, however, painstaking examination reveals varying amounts of heterogeneous elements.

Seminoma and arrhenoblastoma are therefore considered by one group to be of teratomatous derivation, while the opposing group favors an origin from sex cells of various levels of development. The exponents of the teratomatous origin are called on to account for the common occurrence of homogeneous tumors and the paucity of the occasional inclusions. The advocates of the nonteratomatous nature of these tumors have to explain the occasional inclusions of heterogeneous elements. Yet these concepts do not seem to conflict if one considers that the sex cell origin of teratoma, especially when it arises in the gonads, is well supported (Ewing [1]) and widely accepted.

Perhaps the difficulty in deciding between a teratomatous and a nonteratomatous origin could be avoided if it were kept in mind that in either case the tumor originated from the same matrix, i. e., from the sex cell. Sex cells have the potentiality to differentiate into the various tissues of the body in addition to that of perpetuating themselves. Tumors originating from sex cells therefore may be simply a proliferation of those cells. This simple proliferation may account for many forms of seminoma and arrhenoblastoma which fail to reveal teratomatous elements by the most exacting histologic examination. In other instances some of the sex cells may differentiate further. In those instances the tumors would contain a varying amount of teratomatous tissue.

If the same matrix may give rise to homogeneous tumors as well as to tumors containing

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From the Department of Pathology, University of Arkansas School of Medicine.

1. Ewing, J.: Surg., Gynec. & Obst. **12**:230, 1911.
2. Ewing, J.: Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1940, p. 860.
3. Krock, F., and Wolferman, S. J.: Ann. Surg. **114**:78, 1941.
4. Novak, E., in discussion of Krock and Wolferman,[3] p. 89.
5. Meyer, R.: Am. J. Obst. & Gynec. **22**:697, 1931.

various amounts of teratomatous inclusions, the difference in these tumors will have to be regarded as a superficial one. The occurrence of all transitions from a homogeneous tumor to a growth containing abundant evidence of teratomatous elements would be accounted for. If this concept be correct, then the question of a teratomatous or a nonteratomatous origin of seminoma and arrhenoblastoma cannot be settled.

This realization might serve to overcome the tendency to place tumors sufficiently distinct from a pathologic and a clinical point of view into the vast and vague category of teratoma merely because they occasionally contain heterogeneous structures. It seems preferable to retain those tumors apart.

The concept employed in the analysis of the relationship of seminoma and of arrhenoblastoma to teratoma may well be applied to other tumors revealing occasional heterogeneous elements. It may apply to tumors arising either from the gonads or from other locations if their origin from aberrant sex cells can be supported.

Dysgerminoma of the ovary may need to be considered in this connection. Its microscopic appearance is identical with that of seminoma of the testis. For this reason, the tumor was originally referred to as seminoma of the ovary until Meyer in 1931 introduced the term "disgerminoma." The histologic identity, as well as the fact that both seminoma of the testis and dysgerminoma of the ovary are devoid of endocrine manifestations, points to their origin from closely related primitive germinal cells. Dysgerminoma is more commonly associated with teratomatous elements than arrhenoblastoma, according to Novak.[4] However, it is supposed to originate from an undifferentiated germinal cell void of male or female attributes, according to Meyer.[5]

Another tumor to be considered is the Brenner tumor of the ovary, which in the opinion of Plaut[6] arises from the germinal epithelium. However, Dockerty and MacCarty[7] expressed the belief that it represents a one-sided development of teratoma. This belief is supported by its frequent association with pseudomucinous cystadenoma.

It is suggested that certain tumors arising from one developmental level of the sex cell will show an increased tendency to teratomatous differentiation while sex cells in another phase of development will tend merely to reproduce themselves and thereby give rise to homogeneous tumors.

SUMMARY

Sex cells reproduce themselves and differentiate into the various tissues of the body. Tumors arising from sex cells therefore may be homogeneous or may contain varying amounts of heterogeneous structures.

It is generally accepted that seminoma of the testis and arrhenoblastoma of the ovary arise from sex cells. Both show all transitions from a homogeneous tumor to teratoma. The controversy as to their teratomatous or nonteratomatous origin therefore seems futile.

This concept is discussed as applying to other tumors which occasionally show heterogeneous tissues, especially when these tumors arise from the gonads.

6. Plaut, A.: Arch. f. Gynäk. **153**:97, 1933.

7. Dockerty, M. B., and MacCarty, W. C.: Am. J. Obst. & Gynec. **37**:703, 1939.

TOXICOPATHOLOGIC STUDIES ON S-METHYLISOTHIOUREA

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S-methylisothiourea sulfate was recently introduced by Smirk as a vasopressor agent and recommended for use in the treatment of acute circulatory collapse. The pharmacologic properties of this substance and several derivatives have been studied by a number of research workers.[1] It has been mentioned as one of the numerous goitrogenic substances discovered during the last few years.[2] Because of its prolonged and pronounced action in raising blood pressure it has been used with solutions of methyl cellulose in the treatment of experimental histamine shock in dogs.[3] In view of its potential medicinal use in man, experimental toxicopathologic investigations were undertaken by us.

EXPERIMENTAL PROCEDURE

The S-methylisothiourea sulfate used in the experiments was obtained from the Eastman Kodak Company. Dogs, cats, rabbits and rats were exposed to single and repeated doses of this agent, given intravenously and orally. For intravenous administration a 5 per cent aqueous solution was employed and was given either in its natural acid state (p_H 4.4) or in the neutralized state following addition of a solution of sodium hydroxide. It was given orally by capsule to dogs and as a 10 per cent solution was injected into the stomachs of rats through a blunted needle.

OBSERVATIONS AFTER INTRAVENOUS ADMINISTRATION

Dogs, cats, rabbits and rats were used. In dogs the jugular vein, in cats the femoral veins, in rabbits the marginal vein of the ear and in rats the marginal lateral vein of the hindleg were injected.

Dogs.—(*a*) Reactions after a single injection: For the study of acute hematic responses 6 dogs (series 1) ranging in weight from 7.6 to 11.5 Kg., received a single injection of 25 mg. per kilogram of body weight in a 5 per cent aqueous solution. Three of them received a neutralized solution and 3 an acid one. A seventh dog received 50 mg., an eighth dog 75 mg., and a ninth dog 100 mg. per kilogram. Directly before and one, two, five and twenty-four hours after the injection the blood sugar was determined (Folin-Wu method; Lumitron [photoelectric colorimeter]) in the 6 dogs receiving 25 mg. per kilogram (series 1). The hematic studies in the other 3 dogs (series 2) were limited to determinations of the numbers of erythrocytes and leukocytes, the volume of packed cells and the viscosity of plasma. The observations made are summarized in tables 1 and 2.

The data in table 1 indicate that in the dose given S-methylisothiourea has no demonstrable effect on the blood sugar level. From the data in table 2 it appears as if large doses (100 mg. and 75 mg. per kilogram) of the substance cause an early moderate transitory increase in plasmatic viscosity and perhaps also minor hemoconcentration.

(*b*) Reactions after reported injections: As the dog which received 100 mg. per kilogram died within sixty minutes after the injection, it was replaced by a new dog for the subsequent study of the responses elicited by repeated intravenous injections of S-methylisothiourea, and the 5 survivors of the first series were also used. These 6 dogs received daily intravenous injections of 25 mg. per kilogram of the test substance. Three dogs were given the acid solution, while the other 3 dogs received the neutralized solution. One dog of the group given the acid solution died after twenty-eight injections; the 5 survivors were killed after having been given ninety-four injections within four months. The following hematic data were determined: the amount of hemoglobin, the numbers of erythrocytes and leukocytes, the speed of erythrocytic sedimentation, the viscosity of the plasma, the level of the serum cholesterol, the level of the blood sugar. The tests were made at weekly intervals. The results of this investigation are summarized in table 3.

The 3 dogs comprising series 2, weighing from 8.0 to 8.5 Kg., were given daily 50 mg. per kilogram. One dog died after seventeen injections given within seventeen days, while the surviving 2 dogs were given thirty-eight injections within six weeks. They were then killed by an intravenous injection of a 4 per cent formaldehyde solution. The hematic studies in this series were limited to the number of erythrocytes, the number of leukocytes, the volume of packed erythrocytes and the viscosity of the plasma. The hematic reactions observed in the animals of series 2 were in general identical with those of series 1.

The data recorded in table 3 indicate that the hemoglobin showed a general though minor trend toward increased values during the course of the experiment. The number of erythrocytes and that of leukocytes did not undergo any consistent changes but fluctuated considerably within normal limits. The viscosity of the plasma was somewhat lower toward the end of the experimental period than it was originally. Serum cholesterol and blood sugar values moved within normal limits.

From the Warner Institute for Therapeutic Research.

1. Smirk, F. H.: Brit. M. J. **2**:510, 1941. Smirk, F. H., and McGeorge, F. H.: Lancet **2**:301, 1942. McGeorge, M.; Sherif, M., and Smirk, F. H.: J. Physiol. **100**:474, 1942. Fastier, F. N., and Smirk, F. H.: ibid. **101**:379, 1943. Ichniowski, C. T., and Lischer, C.: Pharmacology and Toxicology of Some Aliphatic and Aromatic Isothiourea Compounds, to be published.

2. Astwood, E. B.: J. Pharmacol. & Exper. Therap. **78**:78, 1943.

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The autopsies performed on the 6 dogs of series 1, which were killed by an intravenous injection of air, and on the 3 dogs of series 2, two of which were killed by an intravenous injection of formaldehyde solution, showed no appreciable abnormalities except in 1 dog of series 1. The latter, which died during the course of the experiment, revealed edematous and hemorrhagic lungs and some coagulated blood adherent to the gastric mucosa. The thyroid glands of all the dogs were of small to normal size, red brown to pale brown and firm, and reflected light from their surface.

Histologic studies were made of the following organs: brain, hypophysis, thyroid and parathyroid glands, lungs, heart, aorta, carotid arteries, iliac arteries, pulmonary artery, celiac artery, inferior vena cava, liver, pancreas, stomach, intestine, spleen, adrenal glands, kidneys, prostate gland, testes, urinary bladder, marrow of sternum, thymus, periadrenal ganglion. The sections were stained with hematoxylin and eosin. Sections of the liver were stained for glycogen with Orth's lithium carmine stain. While this was done with ordinary formaldehyde-fixed liver tissue in the case of series 2, in that of series 1 unfixed liver tissue was used. Pieces of the liver were kept in a refrigerator for periods up to seven days. Slices were taken from this tissue daily and sectioned for the determination of the absolute amount of glycogen and the time necessary for its disappearance. Sections prepared from

TABLE 1.—*Reaction of the Blood Sugar Level After a Single Injection of a Solution of S-Methylisothiourea Sulfate*

Group	Dog	Amount of Sugar in Milligrams per Hundred Cubic Centimeters of Blood at Given Time After Injection				
		0	1 Hr.	2 Hr.	5 Hr.	24 Hr.
Acid solution	1350	135	151	146	135	145
	1351	149	149	142	158	154
	1361	140	140	135	112	130
Neutral solution	1362	135	143	118	128	133
	1364	126	...	118	126	130
	1368	145	140	114	120	145

TABLE 2.—*Hematic Reactions After a Single Injection of a Solution of S-Methylisothiourea Sulfate*

Observation	Dog	Dose of Sulfate, Mg.	Value at Given Time After Injection							
			0	5 Min.	15 Min.	45 Min.	60 Min.	120 Min.	240 Min.	24 Hr.
Erythrocytes (millions per cu. mm.)	1170	100	7.3	8.5	9.45	8.3	+			
	1171	75	7.3	8.7	6.25	8.4	6.8	7.65	6.45	6.55
	1181	50	7.5	7.25	7.9	8.8	7.7	8.05	7.05	6.55
Leukocytes (thousands per cu. mm.)	1170	100	14.2	14.4	20.2	17.6	+			
	1171	75	18.0	18.8	15.2	17.4	14.6	17.8	20.4	13.8
	1181	50	9.0	12.1	11.0	9.8	8.0	15.2	11.2	10.1
Viscosity of plasma (centistokes)	1170	100	2.0	1.95	2.15	2.4	+			
	1171	75	2.15	2.15	2.35	2.35	2.45	2.25	2.08	1.8
	1181	50	1.8	2.03	1.85	1.75	1.75	1.65	1.65	1.75

TABLE 3.—*Hematic Reactions After Repeated Injections of a Solution of S-Methylisothiourea Sulfate*

Observation	Dog	Test Solution	Value at Given Interval After Start of Injections								
			1 Wk.	2 Wk.	3 Wk.	5 Wk.	7 Wk.	8 Wk.	9 Wk.	12 Wk.	14 Wk.
Hemoglobin (Gm. per 100 cc.)	1350	Acid solution	18.0	17.1	17.1	20.2	18.2	21.7	19.9	21.7	20.2
	1351		16.3	17.6	18.3	20.4	18.5	18.0	19.7	17.7	19.0
	1361		18.2	18.7	18.2	+					
	1362	Neutral solution	18.7	17.9	18.25	16.3	15.3	16.3	16.8	18.2	19.0
	1364		16.9	15.9	15.8	15.4	19.9	16.6	14.4	14.85	16.3
	1368		14.8	14.4	14.4	17.3	14.65	14.8	17.7	16.9	19.2
Erythrocytes (Millions per cu. mm.)	1350	Acid solution	5.6	7.4	10.0	7.7	9.8	8.5	9.8	10.4	5.8
	1351		8.0	9.0	7.3	8.8	7.1	7.8	9.6	8.1	8.4
	1361		7.6	7.8	7.5	+					
	1362	Neutral solution	8.0	9.2	6.5	5.8	5.7	7.7	7.3	8.2	8.6
	1364		7.0	6.9	6.0	6.2	8.6	6.5	8.6	7.2	7.0
	1368		7.1	7.4	4.6	5.7	7.2	7.6	8.8	8.2	8.7
Leukocytes (thousands per cu. mm.)	1350	Acid solution	16.1	28.6	19.2	17.8	5.8	13.8	14.2	20.8	9.0
	1351		14.8	17.0	18.4	16.4	14.0	19.4	13.8	16.8	13.4
	1361		10.8	13.1	52.4	+					
	1362	Neutral solution	11.2	15.2	17.8	16.2	14.4	10.0	8.2	10.2	8.4
	1364		32.0	37.0	15.8	9.6	7.6	11.6	10.7	11.0	10.4
	1368		25.2	18.0	22.0	12.4	11.2	7.0	5.8	9.8	13.0
Viscosity (centistokes)	1350	Acid solution	2.05	2.0	1.8	1.6	1.9	1.7	2.0	1.85	1.7
	1351		2.25	1.8	1.7	1.7	1.8	1.8	2.0	1.7	1.7
	1361		2.2	1.9	2.0	+					
	1362	Neutral solution	1.95	1.8	2.1	1.7	1.9	1.6	1.9	1.75	1.7
	1364		2.3	2.2	2.1	1.6	1.9	1.6	1.8	1.6	1.7
	1368		2.0	2.2	2.25	1.7	2.0	1.8	1.9	1.7	1.7
Cholesterol (mg. per 100 cc.)	1350	Acid solution	193	104	178	197	198	125	202	190	194
	1351		204	104	240	213	206	112	215	192	259
	1361		248	94	277	+					
	1362	Neutral solution	237	119	173	213	210	135	211	209	224
	1364		218	94	183	208	153	104	181	180	237
	1368		262	124	173	148	193	204	212	212	235
Sugar (mg. per 100 cc.)	1350	Acid solution	163	153	183	168	111	165	165	167	224
	1351		149	153	144	163	158	151	179	182	179
	1361		160	141	191	+					
	1362	Neutral solution	118	122	118	158	132	144	164	181	169
	1364		121	130	128	149	137	139	149	158	167
	1368		116	112	135	144	146	160	139	149	176

liver tissue fixed in absolute alcohol directly after death served as standards and controls.

The brain disclosed no abnormalities except for moderate proliferation of the arteriolar endothelial cells in 1 dog.

The hypophysis showed a normal posterior lobe. The anterior lobe consisted predominantly of eosinophilic cells.

and either filled with thin, liquid, faintly stained and vacuolated colloid or empty. In 1 instance small empty cysts were present which were lined by cylindric and occasionally by papillary epithelium (fig. 1). None of the glands showed diffuse change into a hyperplastic thyrotoxic goiter.

The ascending aorta often exhibited moderate to extensive mucoid imbibition of the inner media. Small

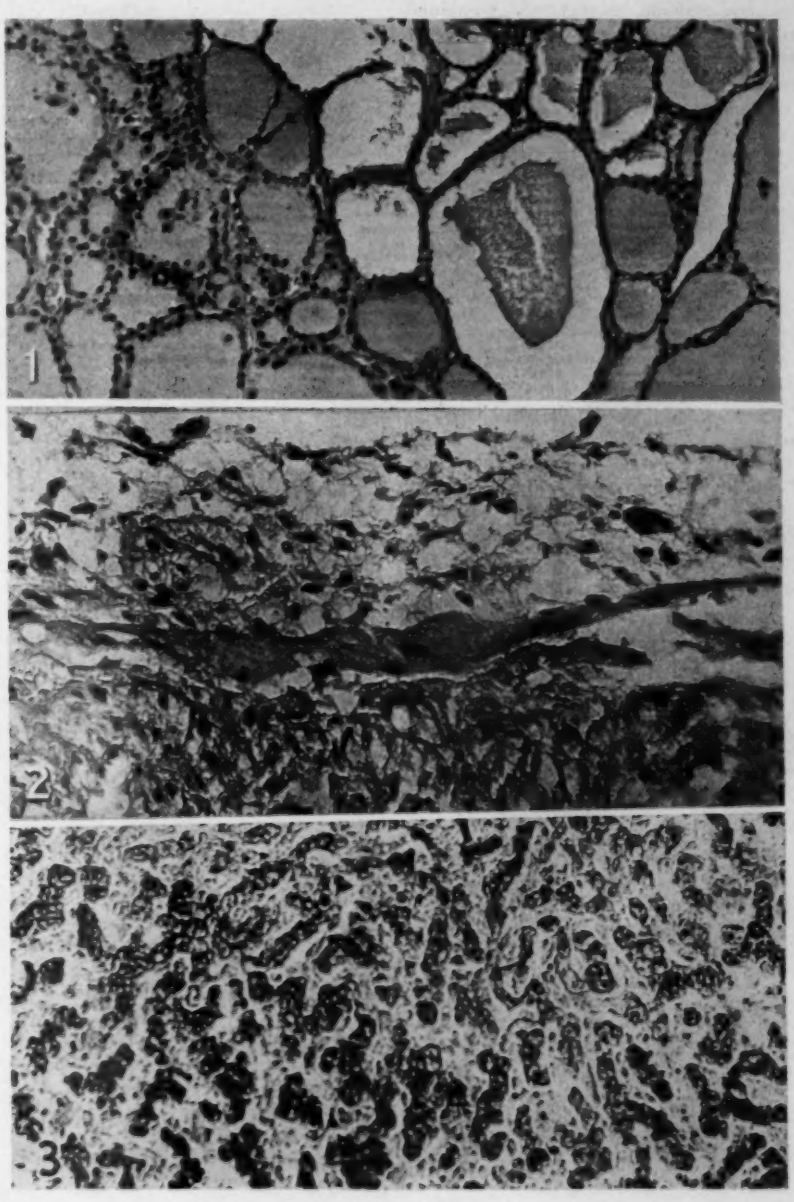

Fig. 1.—Thyroid gland of a dog showing follicles with solid colloid and follicles without or with traces of colloid and desquamated cuboidal epithelial cells.

Fig. 2.—Edematous intimal thickening containing a multinucleated giant cell in the aorta of a dog.

Fig. 3.—Liver of a dog stained with Best's carmine, showing the dark glycogen granulation of the liver cells.

In the thyroid gland the majority of the follicles, which were of medium to large size, were lined by flat epithelium and filled by distinctly red stained solid colloid. One third of the follicles or less were either small or medium sized, lined by low cuboidal epithelium

to moderately large hyaline areas in the outer media of the aortic bulb were present in 3 dogs. The intima of 1 dog revealed a small cushion-like thickening of an albuminoid character. In a second dog there was a larger area of intimal thickening composed of oligo-

cellular edematous tissue containing a few scattered multinucleated giant cells (fig. 2). In a third dog the intima exhibited small blisters and coarse fibrillar thickenings. The thoracic and the abdominal aorta were essentially normal in all dogs.

The carotid artery and the pulmonary artery of 1 dog contained small blister-like edematous thickenings of the intima.

The liver revealed moderate to intense pericentral or diffuse congestion and edema, sometimes combined with atrophy of the pericentral liver cells. In several instances the hepatic cells were rather large and had vacuolar or granular cytoplasm. In 2 of the 8 cases in which the liver was studied for glycogen content, sections stained with Orth's lithium carmine showed liver cells densely crowded with red granules (in 1 of these 2 cases the liver was fixed in formaldehyde solution), in 4 moderate amounts of glycogen were present, while in 2 the liver was practically free from glycogen. The glycogen was demonstrable for seven days in 1 of the 2 cases in which the liver contained large amounts of this substance (fig. 3), at which time appreciable amounts were still present. In the other 4 cases in which daily studies were made the glycogen disappeared from the liver tissue within three days.

All other tissues examined were essentially normal.

Cats.—(*a*) Reactions after a single injection: Four groups of 4 cats each of both sexes were used to study the acute reactions following intravenous administration of a single dose of s-methylisothiourea, injected as a 2.5 per cent aqueous solution (p_H 4.55) into the femoral vein. The animals ranged in weight from 0.85 to 1.8 Kg. The results are summarized in table 4.

TABLE 4.—*Acute Reactions After a Single Injection of a Solution of S-Methylisothiourea Sulfate*

Cats	Dose of Sulfate, Mg. per Kg.	No. of Injections	Percentage Dying Within 24 Hr.	Comment
1 to 4	100	3	75	3 died overnight; 1 died 5 weeks after administration
5 to 8	150	3	75	3 died within 1 hour; survivor was killed 6 weeks later
9 to 12	175	4	100	3 died within 1 hour; 1 died overnight
13 to 16	200	4	100	2 died within 1 hour; 2 died overnight

TABLE 5.—*Reactions After Repeated Injections of a Solution of S-Methylisothiourea Sulfate*

Cat	Weight, Kg.		Dose of Sulfate, Mg. per Kg.	Number of Doses Injected	Total Dose, Gm. per Kg.
	Initial	Final			
1	1.9	1.55	50	20	1.00
2	1.53	1.47	100	28	2.80
3	1.60	....	200	1	0.2

Cat 3 died within one hour after the first injection.

(*b*) Reactions after repeated injections: Three cats, 2 of which were survivors of the acute experiment, were given 6 injections a week, the femoral vein and a 5 per cent aqueous solution of s-methylisothiourea (p_H 4.40) being used. The chronic study began twelve days after the acute experiment and was continued until death of the cats. The results are summarized in table 5.

Autopsies were performed on 12 of the 14 cats which died after a single dose of s-methylisothiourea. The bladder, the small intestine and the spleen were contracted, the heart was dilated, and the lungs were emphysematously distended. The liver was congested. There was some blood-tinged fluid in the pericardial sac of 1 cat which died overnight.

TABLE 6.—*Toxicity of One Intravenous Dose of a Solution of S-Methylisothiourea Sulfate in Rabbits*

Rabbits	Dose of Sulfate, Mg. per Kg.	Result
1 to 3	100	All died within 2 minutes after the injection
4 to 6	50	Two died within 10 minutes, a third one hour after injection
7 to 9	25	All 3 survived

TABLE 7.—*Blood Sugar Level After a Single Injection of a Solution of S-Methylisothiourea Sulfate*

Rabbit	Amount of Sugar in Milligrams per Hundred Cubic Centimeters of Blood at Given Time After Injection				
	Before	1 Hr.	2 Hr.	5 Hr.	24 Hr.
1	177	219	203	182	177
2	177	214	203	177	154
3	159	166	203	168	168
4	168	191	196	168	168
5	141	168	177	166	159

The postmortem examination of the 3 cats which had received repeated injections of the test substance revealed congested lungs in 2 and in 1 cat a lung with numerous large yellow indurated pneumonic patches. The bladder of 1 cat was firmly contracted, as was the left ventricle of the heart. The liver of 1 cat had numerous yellow areas.

The histologic study included the following organs: brain, heart, lungs, aorta, pulmonary artery, liver, pancreas, spleen, adrenal glands, kidney, bladder. There were a few perivascular hemorrhages in the brain of 1 cat. One lung showed hyperemic and atelectatic areas; a second lung contained large pneumonic areas and had purulent bronchitis. The intrapulmonary arteries of the third cat exhibited highly thickened walls with muscular hyperplasia of the media and proliferation and hyalinization of the intima (fig. 4). The liver with the yellow areas revealed complete necrosis of the liver cells. Scanty glycogen granules were demonstrable in 2 livers fixed in formaldehyde. Two pancreases showed scattered interstitial leukocytic infiltrations associated in 1 with focal necroses of the parenchyma. All other organs were essentially normal.

Rabbits.—(*a*) Reactions after a single injection: Three groups of 3 female rabbits each, ranging in weight from 2.4 to 4.5 Kg., were used for the study of the toxicity of s-methylisothiourea administered by the intravenous route. The 5 per cent aqueous s-methylisothiourea solution was adjusted to a p_H of 6.8 to 7.1 by addition of a 1 per cent sodium hydroxide solution. The experimental conditions and the results are summarized in table 6.

This table shows that the minimal lethal dose of S-methylisothiourea sulfate given by the intravenous route is for rabbits in the neighborhood of 50 mg. per kilogram of body weight.

In a second experiment 5 rabbits, weighing from 2 to 3.55 Kg., were given intravenously 25 mg. of S-methylisothiourea sulfate as a 5 per cent solution. The blood sugar was determined directly before the injection and

one, two, five and twenty-four hours later. The rabbits were starved for two days before the injection. The results are presented in table 7.

There was a minor but probably insignificant increase in the blood sugar during the one to two hour period after the injection.

(*b*) Reactions after repeated injections: The effects of repeated intravenous injections of S-methylisothiourea were determined on two series of rabbits. The first series consisted of the 3 rabbits which survived the injection of 25 mg. per kilogram in the acute toxicity experiment. These rabbits received during a period of fifteen weeks ninety-eight intravenous injections of 25 mg. per kilogram of S-methylisothiourea sulfate. At the end of this period they were killed by an intravenous injection of a 4 per cent formaldehyde solution after having received a total of 2.45 Gm. of the test substance per kilogram of body weight. At autopsy the organs of these animals were grossly normal. There was present a large amount of retroperitoneal and peritoneal fat tissue, confirming anatomically the observation of a moderate increase in weight, from an average of 3.4 Kg. to an average of 3.75 Kg., during the experimental period.

The second series was comprised of the 5 rabbits used in the study of the reaction of the blood sugar level to a single injection of 25 mg. per kilogram of S-methylisothiourea. The injections were continued daily for

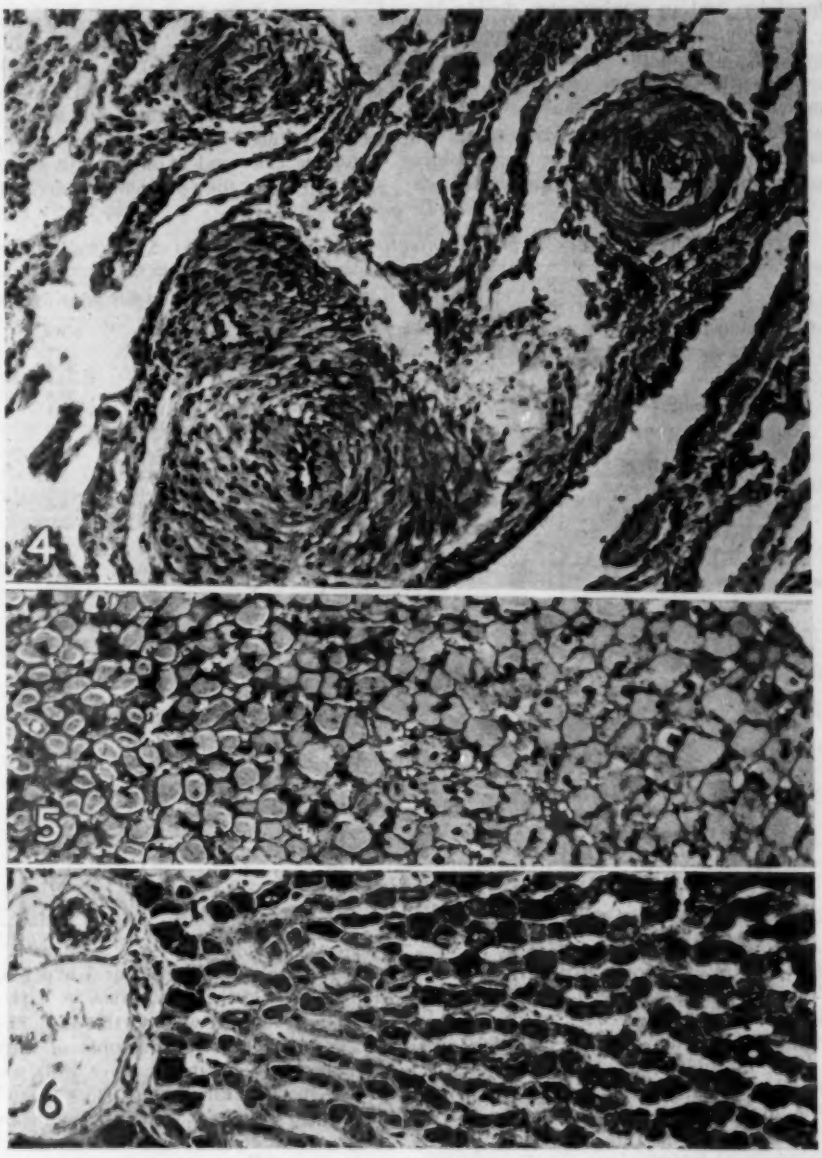

Fig. 4.—Muscular medial hyperplasia and cellular intimal thickening of the pulmonary arteries in a cat.

Fig. 5.—Liver of a rabbit stained with hematoxylin and eosin, showing the vegetable cell-like appearance of the cells containing glycogen.

Fig. 6.—The same liver as in figure 5 stained with Best's carmine to show the dark red-stained glycogen content of the liver cells.

a period of sixty-six days, at the end of which the rabbits were killed by an intravenous injection of ether. The following data on the blood were determined at weekly or biweekly intervals: the hemoglobin content, the numbers of erythrocytes and leukocytes, the sedimentation rate, the plasma viscosity, the level of blood sugar, the level of serum cholesterol. The observations made are contained in table 8.

The data in table 8 indicate that there was a mild increase in hemoglobin and in erythrocytes during the second to fifth week of the experiment, followed by a minor decrease to the range of the original level. After two to three weeks the leukocyte count showed a moderate to marked tendency toward leukopenia. The viscosity of the plasma and the sedimentation rate (not recorded in the table) did not fluctuate appreciably. The blood sugar values remained without definite

The medium-sized pulmonary arteries of 3 rabbits revealed definite thickening of walls caused by muscular hyperplasia and some intimal hyalinization.

The heart of 1 rabbit showed scattered fibrous scars in the myocardium and myocardial arteries with swollen and irregularly cellular walls. The hearts of the other rabbits were normal.

In the aorta of 1 rabbit there was a small oval medial calcification surrounded by circularly arranged muscle cells.

In hematoxylin-eosin–stained sections the liver of 3 rabbits belonging to the second series appeared normal. The livers of the other 5 rabbits showed swollen, polygonal, pale-stained liver cells with sharply outlined cellular membranes and almost unstained, homogeneous central inclusions. The cells closely resembled vegetable cells and exhibited this character particularly strikingly in the pericentral area (fig. 5). There

TABLE 8.—*Hematic Reactions After Repeated Injections of a Solution of S-Methylisothiourea Sulfate*

Observation	Rabbit	Value at Given Interval After Start of Injections						
		1 Wk.	2 Wk.	3 Wk.	5 Wk.	6 Wk.	8 Wk.	9 Wk.
Hemoglobin (Gm. per 100 cc.)	22	14.4	15.4	17.3	14.9	15.4	14.4	14.4
	23	13.9	15.6	17.8	14.0	15.4	14.4	14.4
	24	13.4	15.4	18.0	14.0	13.8	13.0	12.0
	25	14.7	15.9	19.7	15.9	16.1	15.4	14.9
	27	14.2	14.8	18.0	14.0	15.0	13.7	13.9
Erythrocytes (millions per cu. mm.)	22	7.0	5.6	5.6	5.8	8.1	7.6	5.9
	23	5.5	4.2	5.1	5.2	5.8	5.9	4.8
	24	5.3	6.3	4.8	7.1	6.5	5.4	4.7
	25	5.9	6.4	7.6	7.3	8.4	6.4	6.1
	27	5.3	4.3	6.1	6.7	5.0	4.3	4.5
Leukocytes (thousands per cu. mm.)	22	12.8	10.4	6.2	8.8	9.2	5.6	8.0
	23	12.8	6.8	3.2	5.6	6.0	8.4	5.2
	24	12.2	3.6	4.9	8.2	7.2	7.4	6.1
	25	14.8	11.6	9.4	9.2	10.8	11.8	8.3
	27	9.2	6.0	4.2	7.0	5.0	7.8	5.2
Viscosity of plasma (centistokes)	22	1.6	2.1	1.7	1.8	1.5	1.7	1.6
	23	1.8	1.9	1.6	1.8	1.8	1.8	1.7
	24	1.7	1.8	1.7	1.8	1.7	1.7	1.7
	25	1.8	1.8	1.7	1.8	2.0	1.8	1.7
	27	1.9	1.7	1.7	1.6	1.7	1.6	1.7
Blood sugar (mg. per 100 cc.)	22	154	147	161	168	168	149	155
	23	191	156	149	175	140	137	140
	24	178	121	160	202	165	174	178
	25	165	144	142	191	158	165	172
	27	151	131	153	177	193	144	151
Serum cholesterol (mg. per 100 cc.)	22	62	141	163	139	117	213	145
	23	85	138	245	238	190	188	239
	24	77	136	202	134	119	94	115
	25	134	200	110	148	242	196	124
	27	82	193	139	235	199	144	151

variation during the entire experimental period, whereas the serum cholesterol values exhibited a considerable increase starting with the second week.

The autopsies on the 5 rabbits in general showed normal organs. The thyroid gland was small and pale brown in 4 rabbits and slightly enlarged and red-brown in the fifth. The liver was firm, brown-red and had a shiny surface. All rabbits had an abundant amount of peritoneal fat tissue. Small pieces of liver tissue were fixed in absolute alcohol; other pieces were kept without fixation in the refrigerator and slices were removed each day into absolute alcohol for the demonstration of glycogen with Best's lithium carmine.

Histologic examination of the organs of the 8 rabbits showed the following changes:

Infectious meningoencephalitis with formation of epithelioid cell granulomas was present in 3 rabbits. In the other 5 rabbits the brain was essentially normal.

In all the animals the follicles of the thyroid gland were medium sized to large and filled with solid colloid.

occurred, moreover, pericentral hyperemia and degeneration of liver cells, which had in part lost their nuclei.

Sections stained with Orth's lithium carmine revealed the presence of large amounts of intensely red-stained granular deposits in the liver cells, especially in those in the pericentral region (fig. 6). This distribution gave the sections a mottled appearance of large red areas embedded in a pale network. These changes were especially marked in the 3 rabbits of series 1 and in 2 rabbits of series 2. Sections prepared from the unfixed liver tissue kept in the refrigerator for five days and stained with Best's lithium carmine revealed appreciable amounts of glycogen for a period of three to four days after death.

The other organs (pancreas, spleen, adrenal gland, kidney, uterus, ovary, intestine, stomach, skeletal muscle, bone marrow, thymus) were normal in hematoxylin-eosin–stained sections. Sections of heart, aorta, lung, uterus, bone marrow, brain, kidney, spleen and adrenal gland stained for glycogen showed none. Liver sections stained with congo red and crystal violet for the demon-

stration of proteinic cellular abnormalities were also negative, as were those stained with sudan III for the demonstration of fat.

The main evidence obtained from the histologic study of the organs points to a glycogen storage disease of the hepatic type.

Rats.—(*a*) Reactions after a single injection of a neutralized solution: Four groups of 5 rats each were used for the determination of the approximate single lethal dose of S-methylisothiourea when given as a 2.5 per cent solution of the sulfate neutralized with 1 per cent sodium hydroxide to a p_H of 6.95. The solution was injected into the tail vein. The results are listed in table 9.

TABLE 9.—*Toxicity of a Single Dose of a Neutralized Solution of S-Methylisothiourea Sulfate in Rats*

Rats	Dose of Sulfate, Mg. per Kg.	Survivors in 24 Hr.	Percentage That Died
1 to 5	200	3	40
6 to 10	250	2	60
11 to 15	300	1	80
16 to 20	350	0	100

(*b*) Reaction after a single injection of an acid solution: Ninety-five rats, weighing between 130 and 200 Gm., were used, and the acid 10 per cent aqueous solution of S-methylisothiourea sulfate was injected into the lateral marginal vein of the hindleg. The results as to mortality are presented in table 10.

The minimal lethal dose stands at approximately 300 mg. of S-methylisothiourea per kilogram of body weight for both the acid and the neutralized solution.

Of the total of 95 rats used, autopsies were performed on 43, which died within the first twenty-four hours after the injection. There was a total of 56 acute deaths. The survivors numbered 39. The following observations were recorded.

The lungs were often edematous and almost always contained small or large dark red hemorrhagic areas. The heart was contracted, as was the urinary bladder. The liver was moderately congested. The mucosa of the small intestine was either hyperemic or hemorrhagic in an appreciable number of rats. The medulla of the adrenal gland was dark red.

TABLE 10.—*Toxicity of a Single Dose of an Acid Solution of S-Methylisothiourea Sulfate*

Rats	Dose of Sulfate, Mg. per Kg.	Rats That Died in 24 Hr.	Percentage That Died	Delayed Deaths Up to 6 Days
15	150	3	20	0
10	180	0	0	0
15	200	8	55	2
10	225	6	60	0
15	250	11	70	1
10	275	8	80	0
10	300	10	100	0
10	330	10	100	0

In 2 rats with delayed death a hemorrhagic condition of the lung was found associated with fibrinopurulent pleurisy. The adrenal medulla was dark red, as was the spleen.

A histologic study of the organs of these rats was not made, as the great majority of the rats died within a few minutes to one hour after the injection.

(*c*) Reactions after repeated injections: Twenty-one rats were used in the study of the effects of repeated intravenous administration of a 10 per cent S-methylisothiourea solution. The experimental conditions and the results are apparent from the data presented in table 11.

The minimal lethal dose for repeated daily intravenous injections of S-methylisothiourea sulfate solution in rats is in the neighborhood of 200 mg. per kilogram.

In the rats which died during the experiment autopsy showed edematous, hyperemic and hemorrhagic lungs, a contracted heart, a hyperemic liver, sometimes containing yellow areas, and a gastrointestinal tract with congestion of the subserous vessels. In 1 rat which had received twenty-nine injections of 150 mg. per kilogram, the bladder was enormously distended, measuring 1 cm. by 2 cm., and had a thickened, hyperemic and edematous wall. The mucosa showed hemorrhagic spots and the lumen was filled with yellowish white, pinhead to pea sized, smooth, round phosphate stones. The thyroid gland was small; the gastric and intestinal mucosa was hemorrhagic.

The rats which were killed after prolonged treatment with S-methylisothiourea did not exhibit any gross abnormal organic changes.

TABLE 11.—*Reactions After Repeated Injections of a Solution of S-Methylisothiourea Sulfate*

Rats	Dose of Sulfate, Mg. per Kg.	Doses	Result
7	150	1 × 2; 2 × 6; 1 × 10; 1 × 16; 1 × 29; 1 × 30	All died except the 2 rats which received 29 and 30 injections, which were killed
8	180	6 × 2; 2 × 30	6 died, while 2 which received 30 injections were killed
3	200	1 × 6; 2 × 9	1 died, 2 were killed, as veins were obliterated
2	250	1 × 4; 1 × 6	All died
1	300	1 × 6	Died

The organs of 18 rats were subjected to histologic study.

The meningeal and cerebral vessels were congested and often surrounded by small hemorrhages. One rat showed extensive meningeal hemorrhages, while in a second rat intracerebral perivascular round cell infiltrations and small epithelioid cell granulomas were found. The vascular walls were in general normal with the exception that in 1 case they were partly thickened and hyaline.

The thyroid gland in the majority of the rats contained smaller or larger areas of follicles lined by cuboidal cells which had desquamated in part into a thin liquid colloid. The rest of the gland was composed of medium-sized to large follicles lined by flat cells and filled with solid colloid.

The heart in general showed marked congestion of the myocardial vessels, occasionally giving rise to small hemorrhages. In 2 instances it exhibited scattered fibroblastic foci, while in 1 of these cases there were in addition focal necroses and calcifications of the muscle cells.

The lungs were always more or less seriously congested and often showed extensive hemorrhages and edema. The arterial and arteriolar pulmonary vessels were contracted and in 4 cases revealed thickened

hyperplastic walls and in 1 instance small subendothelial calcifications.

The congestion of the liver was always considerable. The hepatic parenchyma was usually intact.

A marked submucosal edema of the intestine was noted in 1 rat, whereas a diffuse leukocytic infiltration of the mucosa and submucosa with ulcerative defects was observed in a second rat.

The kidneys were often congested. Degeneration of the tubular epithelium was found in 4 instances and was associated in 2 of these with extensive formation of albuminous casts and accumulation of such material in the distended glomerular capsule spaces. Minor subendothelial hyaline deposits were noted in the arterioles in 3 cases.

The medulla of the adrenal gland was often hyperemic and in 1 rat revealed hemorrhages. Extensive cortical and medullary necroses and calcifications were present in a second rat.

The testes of 2 rats showed moderate or scattered degeneration of the spermatogenic tubular epithelium associated with calcium concretions in the lumens.

The other organs (aorta, parathyroid glands, spleen, salivary glands, pancreas, stomach, bone marrow) were essentially normal.

OBSERVATIONS AFTER ORAL ADMINISTRATION

Dogs and rats were used for the oral administration of S-methylisothiourea.

Dogs.—Three female dogs, weighing from 5.9 to 6.8 kg., were placed on experiment to study the effects of single and repeated oral administration of S-methylisothiourea sulfate. The sulfate was mixed with lactose and given in capsules. The initial daily dose was 200 mg. per kilogram of body weight. After five such doses 1 dog died. The surviving 2 dogs when they died had received a total of 19 doses, the last fourteen of which were 150 mg. per kilogram. During the experimental period the dogs sustained a considerable loss of weight, amounting to 760 Gm. and 1,200 Gm. respectively for the two dogs which were in the experiment for nineteen days.

Studies were made of the erythrocytes, the leukocytes, the volume of packed cells and the viscosity of plasma in these dogs. These items were determined after the first administration at intervals of one, two, four and twenty-four hours and weekly thereafter. No consistent or significant changes were noted in their values.

Autopsy showed scattered hemorrhages in the thymus and hyperemia of the lung of the dog which died on the sixth day of the experiment. There were no gross abnormalities in the organs of the dogs which survived for nineteen days.

The following observations were made at the histologic examination of the tissues:

The brain showed occasional perivascular hemorrhages.

The hypophysis had a predominantly eosinophilic anterior lobe.

In the thyroid gland the great majority of the follicles were medium sized or large and filled with solid colloid. In 2 cases scattered areas of irregularly shaped follicles were present; some of these were filled with a thin, vacuolated colloid while others had empty lumens into which masses of proliferated lining epithelial cells were desquamated.

The parathyroid gland was markedly congested.

The lung exhibited scattered hemorrhages in the alveoli.

The ascending portion of the aorta showed a considerable mucoid imbibition of the media, presenting in places a cavernous structure. The muscle cells revealed a sort of flaky rarefaction. Small fibrous and partly hyaline foci were scattered throughout the middle part of the media. These changes were most pronounced in the 2 dogs which were treated for nineteen days.

The liver showed moderate pericentral edema and hyperemia with degeneration of liver cells.

The periadrenal ganglion presented striking vacuolation.

The adrenal gland of 1 dog exhibited extensive medullary hemorrhage.

The other organs (heart, large elastic and muscular arteries, pancreas, stomach, intestine, skeletal muscle, bone marrow, pulmonary artery, spleen) were essentially normal.

Rats.—Sets of 5 rats each (weight, 150 to 225 Gm.) received into the stomach 150, 200, 250, 300 and 400 mg. per kilogram of S-methylisothiourea sulfate as a 10 per cent solution, and sets of 10 rats each, 500, 750, 1,000, 1,250 mg. per kilogram. All rats survived a twenty-four hour period except 1 in the group receiving 1,250 mg. which had thus a mortality of 10 per cent. One rat died later in the group given 500 mg. per kilogram and 1 in the group given 1,000 mg. per kilogram.

TABLE 12.—*Reactions After Repeated Oral Administration of S-Methylisothiourea Sulfate*

Rats	Individual Dose, Mg. per Kg.	Number of Doses	Deaths	Survivals
5	250	1 × 14; 1 × 19; 3 × 30	2	3
9	500	2 × 3; 1 × 7; 1 × 9; 2 × 14; 2 × 16; 1 × 25	9	0
9	1,000	4 × 2; 2 × 3; 1 × 4; 1 × 9; 1 × 18	9	0

One month after this experiment the rats of the group which had received 250 mg., 500 mg. and 1,000 mg., respectively, were placed on daily doses of the mentioned sizes. The experimental conditions and results are summarized in table 12.

The organs of the 3 rats which survived 30 intragastric administrations of 250 mg. per kilogram of S-methylisothiourea sulfate and which were then killed were without abnormalities.

The rats which died from the effects of the treatment showed at autopsy: hyperemia of the meningeal vessels; small thyroid glands; hyperemia, edema and hemorrhages of the lungs; contraction of the heart; hyperemia of the liver; congestion of the glandular portion of the gastric mucosa; congestion and often hemorrhages of the intestinal mucosa.

The histologic examination of the organs of 13 of these rats rendered observations closely resembling those made on the rats which received intravenous injections of S-methylisothiourea sulfate solution. There were congestion and hemorrhage in the brain and the lung and congestion of the heart, liver kidney, adrenal gland and spleen. The thyroid gland showed in parts changes indicative of increased functional activity, such as a juicy, cuboidal epithelium desquamating into a thin liquid colloid. The arteries and arterioles of the lungs of 3 rats exhibited hyperplastic and contracted walls lined by crowded endothelial cells

in palisade formation. Testicular degeneration and calcification were found in only 1 rat, while pericarditis was present in 3 rats. Diffuse hemorrhagic imbibition of the mucosa and submucosa of the intestine was recorded for 1 rat.

Similar experimental conditions were used for four series of 5 rats each which received a 1 per cent or a 2 per cent neutralized solution of S-methylisothiourea sulfate. The data in table 13 summarize this experiment.

TABLE 13.—*Reactions After a Single Oral Administration of a Neutralized Solution of S-Methylisothiourea Sulfate*

Rats	Concentration in Solution	Dose of Sulfate, Mg. per Kg.	Mortality in 24 Hr.
5	1%	200	0%
5	1%	400	20%
5	2%	600	80%
5	2%	800	100%

One month later the survivors of this experiment were given daily doses of the same magnitude as those which they had received in the acute experiment. Table 14 summarizes the experimental conditions and results.

TABLE 14.—*Reactions After Repeated Oral Administrations of a Neutralized Solution of S-Methylisothiourea Sulfate*

Rats	Concentration in Solution	Dose of sulfate, Mg. per Kg.	Doses Until Death
5	1%	200	2 × 7; 1 × 8; 1 × 12; 1 × 17
5	1%	400	2 × 2; 1 × 9; 1 × 12
1	2%	600	1 × 12

The total amount of the drug given varied for the individual rats between 0.8 Gm. and 7.2 Gm. per kilogram.

The animals of these experiments showed at autopsy the following abnormalities.

Most of the rats which received 600 mg. and 800 mg. per kilogram and which died within two hours after the first administration of S-methylisothiourea sulfate had marked bilateral hydrothorax with complete collapse of the hyperemic and sometimes hemorrhagic lungs. In others the lungs were definitely congested and contained hemorrhagic areas. The left side of the heart was contracted. The liver was hyperemic. The intestinal lumen was filled with a watery content in several cases. The subserous vessels were congested and surrounded by small hemorrhages. The corticomedullary zone of the adrenal gland was dark red.

The rats which died after several administrations of S-methylisothiourea sulfate solution also exhibited in part moderate to marked bilateral hydrothorax (present in 3 of 10 rats examined). One additional rat showed a thick fibrinous coating of the pleura. One rat showed also a hydropericardium with fibrinous precipitation on the epicardium. The lungs of the rats were hyperemic and usually contained extensive hemorrhages. The heart was contracted. The liver was congested.

Histologic examination of 12 of these rats showed in addition to the congestive and hemorrhagic lesions noted in the previous groups of rats not only hyperplastic thickening of the walls of the pulmonary vessels associated with vacuolation and localized hyalinizations but in 3 rats more or less extensive subendothelial calcifications in the intima as well. There were large ulcerations in the glandular mucosa of the stomach in 1 rat, while in 2 additional rats the submucosa was edematous, hemorrhagic and infiltrated with leukocytes.

COMMENT

The data reported indicate that different species vary considerably in their sensitivity to S-methylisothiourea. Of the species studied, rabbits have the lowest minimal lethal dose (50 mg. per kilogram of the sulfate) for intravenous administration; dogs and cats have a somewhat higher one (100 to 175 mg. per kilogram), while rats display a considerable resistance to this chemical substance and have a minimal lethal dose of 300 to 350 mg. per kilogram. Similar discrepancies are noted in regard to the toxicity of orally introduced S-methylisothiourea because 1 of 3 dogs died after having received 1,000 mg. per kilogram of S-methylisothiourea sulfate in individual doses of 200 mg. per kilogram on five successive days, whereas all rats which were given intragastrically a single dose of 1,000 mg. per kilogram survived. If, however, the solution of S-methylisothiourea sulfate is neutralized before intragastric introduction its toxicity is considerably increased, as the single lethal dose of the sulfate is then 800 mg. per kilogram for rats. This increased toxic action of the neutralized solution was also reflected by the pathologic changes observed in the rats thus treated. In many instances they showed marked bilateral hydrothorax and in a few instances also hydropericardium, conditions not seen in rats treated with the acid solution.

After both the single and the repeated intravenous injection of the solution of S-methylisothiourea sulfate there was a tendency toward higher hemoglobin and erythrocyte levels in dogs and rabbits. This reaction is attributable to the vasoconstrictive anoxemia elicited by this vasopressor agent and resembles similar effects elicited in animals by the introduction of epinephrine, ephedrine, benzedrine, posterior pituitary substance and lead, i. e., substances exerting an identical effect on the vascular system.[4] It may be possible that the hyperplastic and sclerotic

4. (*a*) Davis, J. E.: Am. J. Physiol. **134**:219, 1941; **137**:699, 1942; Federation Proc. **1**:19, 1942. (*b*) Davis, J. E., and Harris, A. M.: Am. J. Physiol. **137**:94, 1942. (*c*) Ehrich, W. E., and Krumbhaar, E. B.: Ann. Int. Med. **10**:1874, 1937. (*d*) Hueper, W. C.: Occupational Tumors and Allied Diseases, Springfield, Ill., Charles C Thomas, Publisher, 1942, pp. 557-586; Medicine **20**:397, 1941.

lesions observed in the pulmonary arterial branches of dogs, cats, rabbits and rats have contributed to this reaction by interfering with the proper oxygenation of the blood, especially since arteriosclerosis of the lung is one of the several causes of polycythemia.[5]

With the exception of a few small albuminoid intimal thickenings and blister-like intimal edematous areas of the aorta present in some of the dogs given injections and minor medial degenerations in the orally treated dogs, there was no evidence of any severe or significant damage in the large arteries of any animal of the various species used. This observation is remarkable in view of the fact that the intravenous injection of epinephrine hydrochloride readily elicits severe medial necroses and calcifications of the aorta in rabbits. It deserves additional attention because of the antithyroid action displayed by S-methylisothiourea which in the rabbits resulted in a probably hypothyroidotic increase of the cholesterol content of the blood. This is remarkable as a number of investigators[6] have observed in rats fed with another goitrogenic agent, sulfaguanidine, considerable calcification of the aorta. It is moreover well known that hypothyroidotic myxedematous persons have a high level of blood cholesterol and display a definite tendency toward precocious and severe atherosclerosis (Hueper[4d]), a hematic and vascular reactivity shared by thyroidectomized animals. A plausible explanation for this discrepancy cannot be offered at this time, but it must be pointed out that the animals received intravenously high and often sublethal amounts of the drug over long periods. It may be mentioned, however, that none of the animals showed the extensive or even diffuse hyperplastic transformation of the thyroid gland described by some observers.[7] The pituitary glands of the animals of the present study did not exhibit any noticeable increase in the number of basophilic cells or any disintegration of the eosinophilic elements such as that associated with severe hypothyroidism. The only evidence that thyroid function was impaired was provided by the hypercholesteremia demonstrated in the rabbits and by minor to moderate hyperplastic changes in the thyroid glands of some but not all animals subjected to treatment with S-methylisothiourea for a long period.

It is possible, on the other hand, that the hypercholesteremia does not represent impairment of thyroid function but is related to the disturbance of glycogen metabolism manifested by the hepatic glycogenosis observed in some of the dogs and rabbits. The occurrence of lipoidemia and hypercholesteremia in the presence of a glycogen storage disease has been reported by Beumer,[8] Krakower[9] and Wagner.[10] The glycogen stored in the liver cells of the animals treated with S-methylisothiourea showed the reduced solubility in an aqueous medium (formaldehyde solution) and the increased resistance to postmortem glycogenolysis found in von Gierke's hepatic thesaurosis of glycogen (Seckel[11]). These observations are of distinct clinical significance because of the proposed use of thiourea compounds in the treatment of thyrotoxicosis. They show that the action of such compounds is apparently not limited to inhibition of the production of thyroid hormone but may extend to other organs and functions.

Mention may finally be made of the congestion of the myocardial vessels found in many of the animals examined, as an improved myocardial blood supply may be in part responsible for the beneficial action displayed by S-methylisothiourea in the treatment of shock.

SUMMARY

The minimal lethal intravenous and oral doses of S-methylisothiourea sulfate have been determined in dogs, cats, rabbits and rats. The rabbits were found to be least and the rats most resistant to this chemical.

A neutralized solution of S-methylisothiourea given intragastrically is distinctly more toxic than an acid solution and produces in rats hydrothorax and hydropericardium in addition to inflammatory and hemorrhagic changes in the stomach and the intestine.

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The vasoconstrictive anoxemia action of S-methylisothiourea and possibly also the arteriosclerotic changes elicited in the pulmonary vessels are considered responsible for the mild hemoconcentration observed after single and repeated intravenous injections of solutions of S-methylisothiourea sulfate.

S-methylisothiourea does not cause any appreciable degenerative intimal or medial changes in the aorta and other large arterial vessels, such as those elicited by epinephrine in rabbits, in spite of the fact that it produces in these animals an apparently hypothyroidotic hypercholesteremia and exerts a constrictive action on vascular walls. The hyperplastic, hyperthyroidotic follicular changes of the thyroid gland were of mild to moderate degree even after the administration of large doses over long periods.

Some of the dogs and rabbits given repeated intravenous injections of a solution of S-methylisothiourea sulfate exhibited marked retention of glycogen in the liver cells such as that seen in von Gierke's glycogenosis.

The observations made indicate that thiourea compounds may exert organic and functional effects other than those connected with impairment of the production of thyroid hormone. Some caution should therefore be employed in the prolonged clinical use of these compounds in the treatment of thyrotoxicosis.

DEMONSTRATION OF THE LESION PRODUCED BY EXPERIMENTAL POLIOMYELITIS IN THE CENTRAL NERVOUS SYSTEM OF THE MOUSE

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During the past three years, evidence has been presented that poliomyelitis may be transmitted to mice, and it has been shown that the histologic changes in the central nervous system observed in this species closely resemble those in man.[1] If some important investigators[2] still believe that mouse tissue is so poorly differentiated that it is not suitable for the work on poliomyelitis, this is obviously due to the poor state of development of the methods for the histologic study of the central nervous system of the mouse. It is true that many ganglion cells of this animal have so little cytoplasm that early changes in the Nissl substance are not readily recognizable; however, necrosis of ganglion cells, neuronophagia, gliosis, perivascular lymphocytic infiltration and other alterations are clearly seen if the tissue is properly fixed and stained. Since the mouse is an important experimental animal, particularly for studies in which large numbers of animals are needed, it seemed to be desirable to present a detailed description of, and some results obtained by, the method developed in the Children's Hospital of Philadelphia during the course of studies of the relation of the diet of mice to their resistance to the Lansing strain of poliomyelitis virus.[3]

METHOD

Operating Chamber.—Each mouse is dissected in an operating chamber permitting preparation of the animal and immediate fixation of the tissues at body temperature, as well as protection of the worker from the fumes of the fixative. The chamber (fig. 1) is essentially a bottomless box 37 inches long, 25 inches wide and 32 inches high (94 by 63.5 by 81 cm.), made of 8 ply wood and lined with ¼ inch (6 mm.) sheet asbestos. A plate glass-covered table top over which it is fastened serves as the floor. The set-in top permits easy removal for repairs or photographing of operations.

There are three separate dissecting units, enabling three operators to share the work on one animal or each to work on a different animal at the same time. Each unit has an inset window through which the operator may observe his dissection. Beneath each of these windows are two circular openings (inset, fig. 1), each fitted with a pair of embroidery hoops which hold a sleeve in place. Extending out from the box on a plane with the base of each of the windows is a shelf 4 inches (10 cm.) wide which protects the operator by deflecting the fumes which escape through the armholes.

Each unit is equipped with a 125 cc. separatory funnel attached to a right angle 6 mm. glass tube, for the purpose of dripping fixative onto the exposed tissue. In addition, the unit to the left of the door (fig. 1) has perfusion equipment, which consists of two separatory funnels, one for saline solution and one for fixative, connected by a Y tube.

The spotlight used in one of the units is an ophthalmologist's operating light; in the other two are equally satisfactory and less expensive 8 mm. film projectors (Eastman Kodak Company, kodaslide projectors, model 1). An individual switch enables each operator to turn off his spotlight when it is not in use.

Electric current is brought to a quadruple outlet for the following: (1) four 150 watt light bulbs used to bring the temperature of the chamber up to 38 C., after which they are turned off by a switch on the outside; (2) two 60 watt light bulbs controlled by a thermostat in order to maintain the desired temperature; (3) the projector spotlights and an electric fan to help distribute the heat, and (4) the rheostat for the ophthalmologist's operating light.

A sleeve-protected armhole in the door and another to the left of the door, as well as an oblong window to the left of the door, are used by the person who operates the perfusion apparatus.

Dissection and Fixation.—When only brain and cord are being preserved, the authors find that perfusion does not bring the fixative in contact with the cells as quickly as does rapid evisceration followed by a brief rinse of the surface with isotonic solution of sodium chloride to remove excess blood and then removal of the skull and vertebrae while the fixative is continually dropping on the brain and cord from the time of exposure until dissection is completed. Hence, the following method is recommended: The mouse is taken into the operating chamber (38 C.) and is lightly anesthetized by holding an ether tube over its nose. The anterior portion of the pelvic girdle, the viscera, the ribs to within 3 mm. of the vertebrae, the lower jaw, the eyes and all the soft tissues attached to the outer surface of the floor of the skull are removed.

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1. (*a*) Lillie, R. D., and Armstrong, C.: Pub. Health Rep. **55**:718, 1940. (*b*) Jungeblut, C. W., and Sanders, M.: J. Exper. Med. **72**:407, 1940. (*c*) Wolf, A.: ibid. **76**:53, 1942.

2. Personal communications to the authors.

3. Foster, C.; Jones, J. H.; Henle, W., and Dorfman, F.: J. Exper. Med. **79**:221, 1944.

Skull and vertebrae are opened with the smallest available cuticle nippers (La Cross) while the eviscerated animal is being held in the hand, on its back, by the legs and by the skin. By inserting one blade of the nippers just enough to get under the bone and directing it upward as cutting proceeds, it is possible to avoid trauma to essential tissues. It has been found best to remove the ventral and lateral surfaces of the bony case first, beginning with the tympanic bulla and proceeding forward and laterally first, then medially and downward until the ventral and lateral surfaces of the brain and cord are exposed. Usually no attempt is made to dissect out the perifloccula. Care should be taken not to expose any part of the olfactory bulbs until the ventral and lateral bones of the rest of the skull and the vertebrae (including the sacral) are removed.

Small, curved, round-pointed eye scissors are used to clip any restraining nerves and membranes and to lift the brain and cord gently away from the dorsal portion of the skull and vertebrae, after which they are allowed to drop into a Petri dish containing fixative and to remain there for five minutes. They are then divided with a double-thin razor blade at the optic chiasma, midthalamus, pons, medulla oblongata, cervical swelling, middorsal area, lower dorsal area, lumbar swelling and lower lumbar area. The pieces are placed in a glass tube 1 inch (2.5 cm.) in diameter and 2 inches (5 cm.) long, which is lined with filter paper and surrounded with fine absorbent gauze in a jar of fixative and placed in the refrigerator for twenty-four hours. The tissues remain in these glass tubes until ready for embedding.

The fixative used for the present study is Bouin's fluid with urea,[4] which was prepared as follows:

Picric acid (trinitrophenol) (saturated solution in distilled water)	750 cc.
Neutral 36 per cent formaldehyde solution	250 cc.
Glacial acetic acid	50 cc.
Urea crystals	20 gm.

The urea will be precipitated if it is not added a little at a time during active stirring. With high speed rotation on a Borner rotator, at least five minutes is needed to add the urea to 1,050 cc.

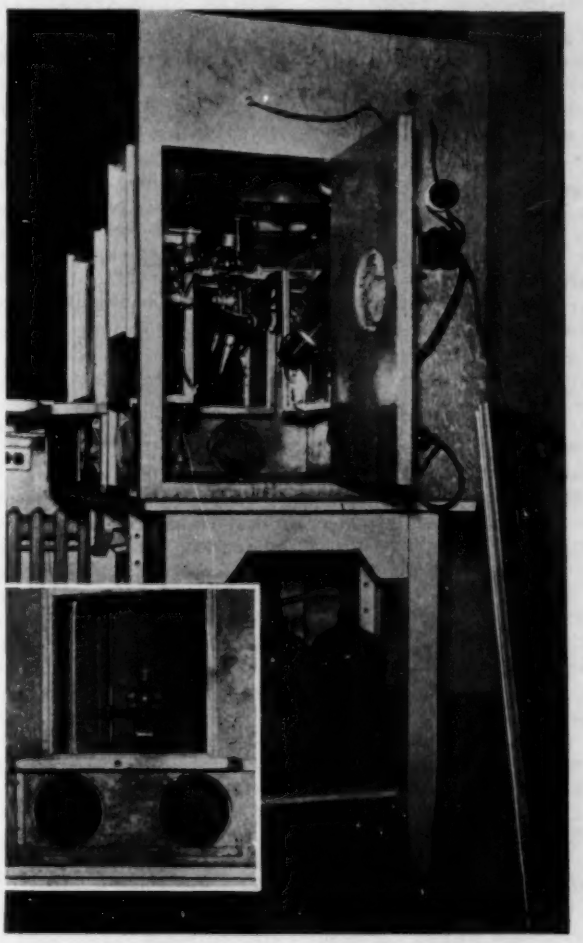

Fig. 1.—The operating chamber. Inset: One operating unit.

Washing.—After proper fixation the tissues are passed consecutively at hourly intervals through 10, 30, 50 and 70 per cent ethyl alcohol. They are then transferred to 70 per cent ethyl alcohol plus 2 per cent of a saturated aqueous solution of lithium carbonate, which is changed at convenient intervals until picric acid (trinitrophenol) is no longer visible after twelve hours'

4. McClung, C. E.: Microscopical Technique, New York, Paul B. Hoeber, Inc., 1937, p. 516.

standing. At this time they are placed in 70 per cent ethyl alcohol, where they may be stored.

Dehydration, Clearing and Infiltration.—Dehydration is accomplished by keeping the tissues for one hour in each alcohol of a normal refined butyl alcohol series [5] which is prepared as follows:

	Water	Alcohols Ethyl	Butyl
1	50	40	10
2	30	50	20
3	15	50	35
4	5	40	55
5	0	25	75
6	0	0	100
7	0	0	100

Following this the tissues are placed for three hours in equal parts of butyl alcohol and melted paraffin (melting point, 53 to 55 C.; a product of the Arthur H. Thomas Company, especially refined for histologic work), and four hours (two changes) in latex-paraffin,[6] which is prepared as follows:

1. Preparation of latex-paraffin stock: Pour liquid latex (Firestone Rubber and Latex Products Company) into an enamel or glass tray in a layer not more than ⅛ inch (3 mm.) thick. Set in a warm, dust-protected place, such as a 37 C. incubator, until it hardens, turns from milky white to amber and becomes semitransparent. This will take several days.

Weigh the latex. Weigh out nineteen times as much paraffin (of the special histologic type described) as there is latex. Melt the paraffin in a water bath kept between 55 and 60 C.

Cut the latex in cubes of not more than ⅛ inch, and drop them one by one into the melted paraffin.

Maintain this at 55 to 60 C., stirring every few hours, for one week. Force any remaining lumps of latex through a fine strainer (the conical Chinese type with 1 mm. holes is useful). Allow the mixture to remain at 55 to 60 C. for a day or two more, until the latex becomes homogeneous with the paraffin. Pour out in a layer about ¼ inch (6 mm.) thick, cool slightly, cut in ½ inch (12.5 mm.) squares and store.

2. Preparation of paraffin ready for infiltration and embedding [7]: The materials needed are 140 parts paraffin (of the special type described), 20 parts latex-paraffin stock and 1 part white beeswax. Melt the paraffin in a water bath kept at 55 to 60 C.; add the latex-paraffin stock. Allow this to melt and become homogeneous with the paraffin. Add the beeswax cut into ½ inch cubes. Allow this to melt and become homogeneous with the paraffin and latex. Filter through cotton.

Embedding.—After infiltration with paraffin the brain and cord of each mouse are embedded so that all nine of the anatomic divisions indicated under "Dissection and Fixation" are represented in each slice of the block.

Embedding boats are formed on a piece of ground glass which rests on the floor of a container large enough to give room for sufficient water and ice to cover completely the block containing the tissues.

One pint (473 cc.) aluminum cups compressed to form a lip are used for both the water and the paraffin. These are poured simultaneously from the two cups so that the water causes the paraffin to form a wall within which the rest of the paraffin remains melted.

If the temperature of the water during embedding is only about 3 degrees cooler than that of the melted paraffin, a very thin wall and base are formed, allowing the embedding of the nine pieces of tissue with their faces at the same level.

Sections are cut at 6 to 8 microns in thickness.

Staining Methods.—The staining methods recommended by us are azure II-eosin, gallocyanin and Bodian's protargol. In addition, hematoxylin-eosin and the stain described by Smith and Quigley [8] may be employed, though the latter gives better results with tissues fixed in a neutral fixative.

Materials Used [8a] for Staining and Their Preparation.—(*a*) Azure II and eosin: For part 1 use 0.1 per cent azure II (only that marketed by National Aniline Division, Allied Chemical and Dye Corporation) in double distilled water; for part 2, 0.1 per cent eosin Y (Coleman & Bell Company) in double-distilled water. The stain consists of 25 cc. of part 1 plus 50 cc. of part 2 plus 200 cc. of double-distilled water. The preparation is usually unsatisfactory after three weeks.

(*b*) Gallocyanin: The materials needed are chromalum (chromium and potassium sulfate) 1.5 Gm., double-distilled water 300.0 cc. and gallocyanin (Hartman-Leddon Co., Inc.: Alizarin blue RBN, fast violet) 0.45 Gm. Dissolve the chromalum in double-distilled water, add dye, mix thoroughly, heat gradually and boil gently fifteen to twenty-five minutes, shaking frequently. Cool gradually and filter.

(*c*) Protargol (strong silver protein) copper solution: Prepare a 1 per cent solution of protargol (Winthrop Chemical Company, Inc.) in double-distilled water. It is important that the protargol be freshly dissolved without stirring, by being scattered on the surface of the water and allowed to remain until it is absorbed. The entire amount may be added at one time and manipulated away from the sides of the beaker with a glass rod, very gently. The solution may be used only once.

For each 100 cc. of protargol solution, add 5 Gm. of copper filings (General Chemical Company, copper metal heavy turnings) cut fine. Allow these to remain in the bottom of the staining dish. Do not let the copper filings come in direct contact with the slides.

(*d*) Reducing solution: Dissolve 1 Gm. of hydroquinone and 5 Gm. of sodium sulfite in 100 cc. of double-distilled water. This must be made up fresh each day.

(*e*) Gold chloride solution: To a 1 per cent solution of gold chloride in double-distilled water add 3 drops of glacial acetic acid for each hundred cubic centimeters. Filter after each use and keep indefinitely.

(*f*) One per cent oxalic acid has been found more satisfactory than the 2 per cent oxalic acid indicated by Bodian.[9]

5. Zirkle, C.: Science **71**:102, 1930.

6. Beyer, E.: Personal communication to the authors.

7. Since batches of latex may differ and the consistency best suited to the requirements of individual workers may vary, it is suggested that test blocks containing varying proportions of the paraffin-latex stock be made.

8. McClung,[4] p. 473.

8a. All chemicals used are chemically pure Baker or Merck "reagent" unless otherwise stated. Solutions of all chemicals for which solids were received in brown bottles are stored in brown bottles.

9. Habel, R. R.: Personal communication to the authors.

(*g*) Cole's iodine-ripened hematoxylin[10]: Dissolve 0.5 Gm. of hematoxylin crystals in 250 cc. of warm water. Add 50 cc. of 1 per cent iodine (in 95 per cent ethyl alcohol). Add 700 cc. of saturated aqueous ammonium-alum solution. Bring the mixture to a boil and allow to cool. Any slight tendency for the cytoplasm to stain can be corrected easily by the addition of small amounts of alum solution.

(*h*) Acid alcohol: Add 0.5 cc. of concentrated hydrochloric acid (37 per cent) to 100 cc. of 70 per cent ethyl alcohol.

(*i*) Diluted solution of ammonia U.S.P.. Keep this solution on a table separated from other parts of the staining series.

(*j*) Alcoholic eosin: Prepare 2 per cent solution of eosin Y (Coleman & Bell Company) in 70 per cent ethyl alcohol.

(*k*) Carbol-xylene: Dissolve 120 Gm. of phenol crystals in 3,320 cc. of xylene.

(*l*) Mordant: Prepare a 4 per cent solution of ferric ammonium sulfate in double-distilled water.

(*m*) Smith and Quigley Hematoxylin: Dissolve 0.3 Gm. of light brown or yellow selected crystals of hematoxylin in 30 cc. of 100 per cent ethyl alcohol diluted with 264 cc. of double-distilled water. Just before using, add 6 cc. of glacial acetic acid.

(*n*) Clarite: A 60 per cent toluene solution of the dried resin (Hartman-Leddon) is used.

Staining Routines Employed.—Separate vessels are used for each step. Letters in parentheses refer to solution described under "Materials Used for Staining and Their Preparation."

a) Azure II and eosin[11]: This stain is used for blood elements and nissl substance.

1. Decerate and hydrate tissues.
2. Place tissue slides in azure II and eosin stain (*a*) for fifteen to sixteen (not more than seventeen) hours. Differentiate one slide at a time, leaving other slides in the stain, because the differentiation time may vary from fifteen seconds to fifteen minutes, depending on the specific reaction of the tissue.
3. Dip in 95 per cent ethyl alcohol. Gently raise and lower slide in alcohol until this runs off in sheets instead of in streaks, to avoid carrying too much stain back to the differentiating bath.
4. Differentiate in 95 per cent ethyl alcohol with continual agitation.
5. Dip in 95 per cent ethyl alcohol as in step 3 to remove the dye from the surface of the slide.
6. Dehydrate, clear and mount.

(*b*) Gallocyanin[12]: This is a stain for nissl substance.

1. Decerate and hydrate.
2. Place tissue slides in gallocyanin stain (*b*) for twenty-four hours at room temperature.
3. Dip in single-distilled water. Gently raise and lower slide in water until this runs off in sheets instead of in streaks.
4. Repeat step 3 in another vessel.
5. Dip in 10 per cent ethyl alcohol as described in step 3.
6. Dip in 30 per cent ethyl alcohol as described in step 3.
7. Dip in 50 per cent ethyl alcohol as described in step 3.
8. Dip in 70 per cent ethyl alcohol as described in step 3.
9. Dip in 83 per cent ethyl alcohol as described in step 3.
10. Dip in 95 per cent ethyl alcohol as described in step 3.
11. Dehydrate, clear and mount.

(*c*) Bodian's Protargol[13]: This stain is used for nerve fibers and endings.

1. Decerate and hydrate.
2. Place slides in protargol-copper solution (*c*) for forty-eight hours at 37 C.
3. Dip in double-distilled water. Gently raise and lower slide in water until this runs off in sheets instead of in streaks.
4. Repeat step 3 in another vessel.
5. Repeat step 3 in another vessel.
6. Place slides in reducing solution (*d*) for ten minutes.
7. Place slides in gold chloride solution (*e*) until generally gray (one to five minutes).
8. Place slides in 1 per cent oxalic acid solution (*f*) until surface shows a purplish cast (one to two minutes).
9. Repeat step 3 in another vessel.
10. Repeat step 3 in another vessel.
11. Place in clear 5 per cent thiosulfate solution in double-distilled water for one minute.
12. Place in clear 5 per cent thiosulfate solution in double-distilled water for three minutes.
13. Place in clear 5 per cent thiosulfate solution in double-distilled water for six minutes.
14. Repeat step 3 in another vessel.
15. Repeat step 3 in another vessel.
16. Dip in 10 per cent ethyl alcohol as described in step 3.
17. Dip in 30 per cent ethyl alcohol as described in step 3.
18. Dip in 50 per cent ethyl alcohol as described in step 3.
19. Dip in 70 per cent ethyl alcohol as described in step 3.
20. Dip in 83 per cent ethyl alcohol as described in step 3.
21. Dehydrate, clear and mount.

(*d*) Hematoxylin and eosin: This stain is used for general cytologic observations.

1. Decerate and hydrate.
2. Place slides in Cole's hematoxylin (*g*) for time sufficient to give the desired effect (one to fifteen minutes).
3. Dip slides in single-distilled water. Gently raise and lower in water until this runs off in sheets instead of in streaks.
4. Repeat step 3 in another vessel.
5. Dip in 10 per cent ethyl alcohol as described in step 3.
6. Dip in 30 per cent ethyl alcohol as described in step 3.

10. Cole, E. C.: Stain Technol. **18**:125, 1943.
11. Brown, D.: Personal communication to the authors.
12. Einarson, L.: Am. J. Path. **8**:295, 1932.
13. Bodian, D.: Anat. Rec. **65**:89, 1936. Habel.[9]

7. Dip in 50 per cent ethyl alcohol as described in step 3.
8. Dip in 70 per cent ethyl alcohol as described in step 3.
9. Place in acid alcohol (*h*) until differentiated and light red.
10. Dip in 50 per cent ethyl alcohol as described in step 3.
11. Dip in 30 per cent ethyl alcohol as described in step 3.
12. Dip in 10 per cent ethyl alcohol as described in step 3.

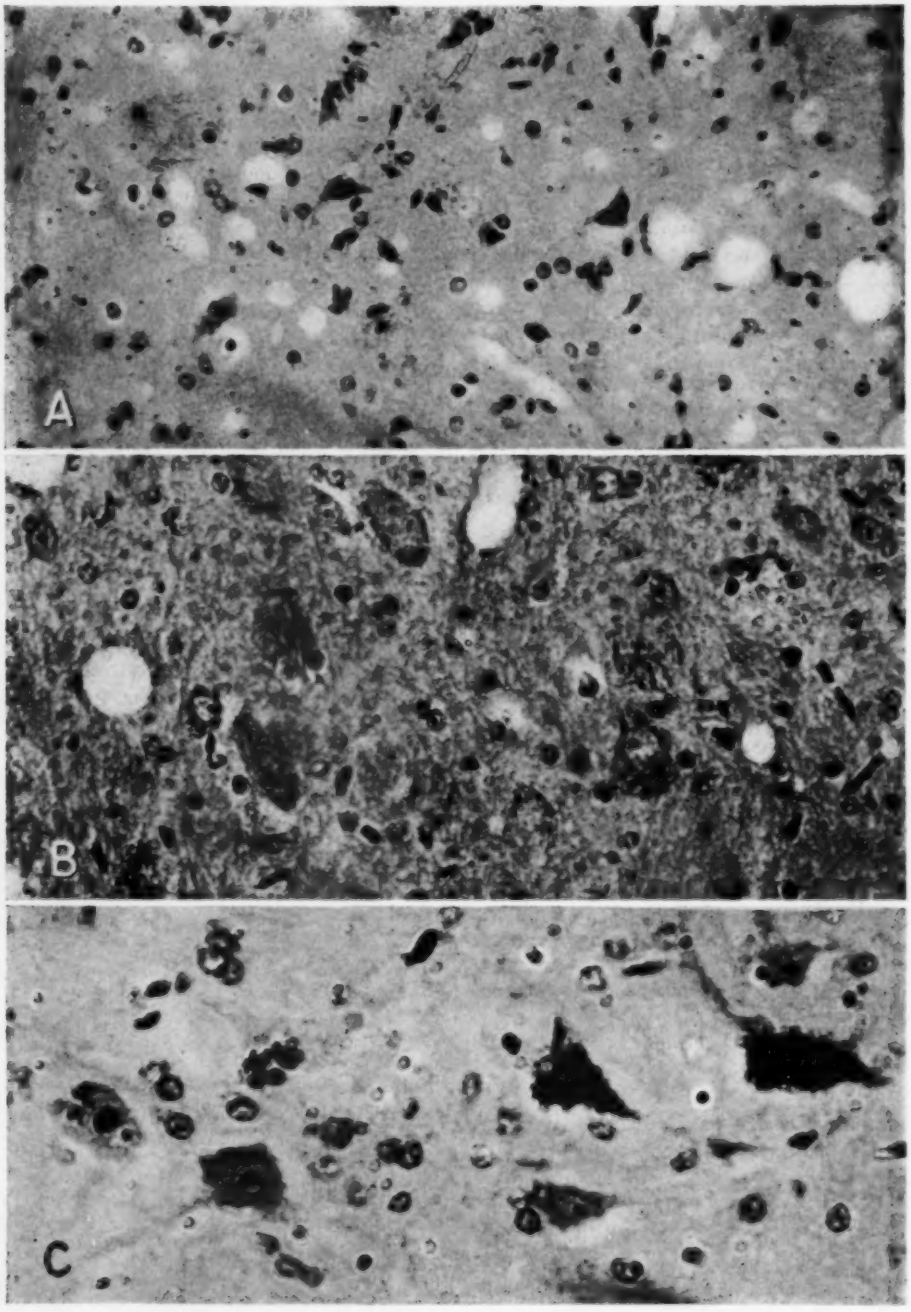

Fig. 2.—*A*, coagulation necrosis of ganglion cells in an anterior horn of the lumbar swelling of the spinal cord of mouse 1. Azure II-eosin. × 391.

B, cytolysis of ganglion cells in an anterior horn of the lumbar swelling of the spinal cord of mouse 2. Azure II-eosin. × 414.

C, necrosis of ganglion cells with invasion by polymorphonuclear leukocytes in an anterior horn of the lumbar swelling of the spinal cord of mouse 1. Azure II-eosin. × 644.

13. Dip in single-distilled water as described in step 3.
14. Repeat step 13 in another vessel.
15. Place in vapor of diluted solution of ammonia U. S. P. (*i*) until blue (one to three seconds). Keep the solution on a separate table.
16. Dip in 10 per cent ethyl alcohol as described in step 3.
17. Dip in 30 per cent ethyl alcohol as described in step 3.
18. Dip in 50 per cent ethyl alcohol as described in step 3.
19. Place in alcoholic eosin (*j*) for time sufficient to give the desired effect (one to three minutes).
20. Dip in 83 per cent ethyl alcohol as described in step 3.
21. Repeat step 20 in another vessel.
22. Make final differentiation in 95 per cent ethyl alcohol.
23. Place in 100 per cent ethyl alcohol for one minute.
24. Place in carbol-xylene (*k*) for ten minutes.
25. Place in xylene for three minutes.
26. Repeat step 25 in another vessel.
27. Mount in clarite (*n*).

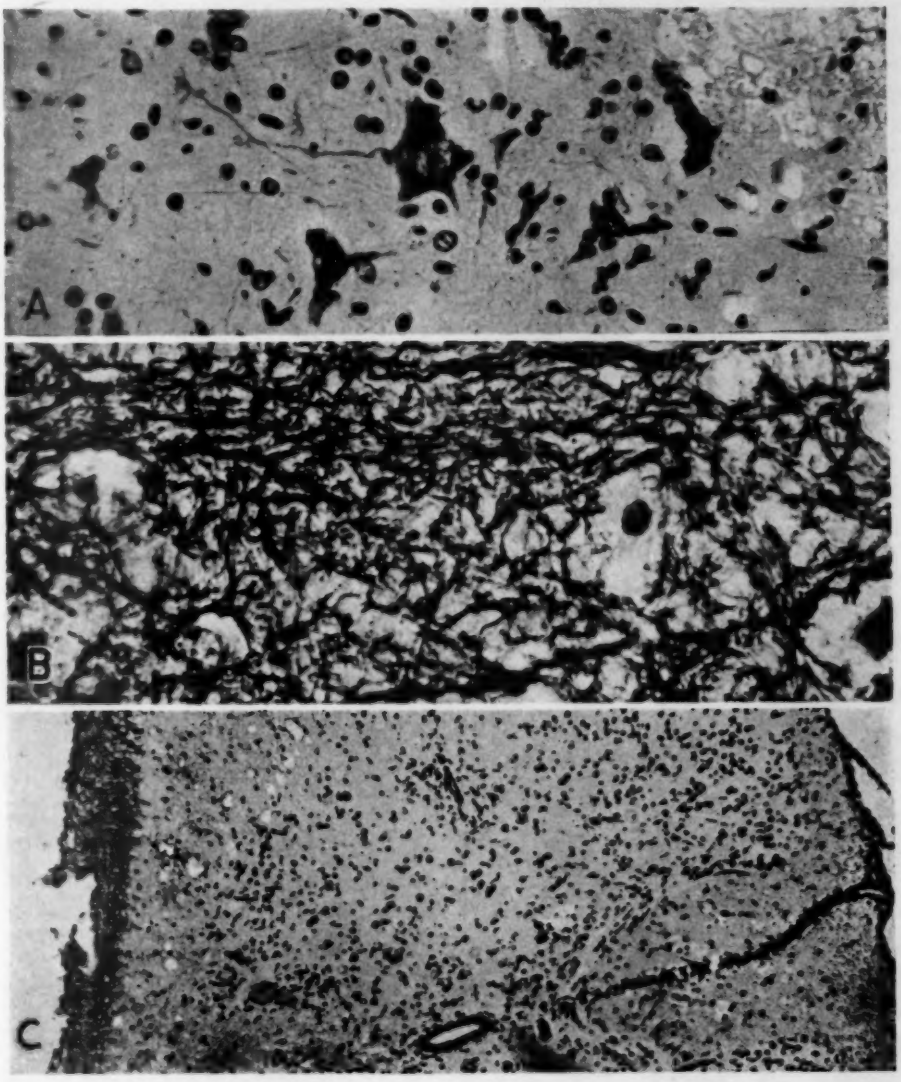

Fig. 3.—*A*, neuronophagia and glial proliferation in an anterior horn of the lumbar swelling of the spinal cord of mouse 1. Gallocyanin. × 391.

B, necrosis of ganglion cells and status spongiosus in an anterior horn of the lumbar swelling of the spinal cord of mouse 1. Bodian's stain. × 736.

C, glial proliferation and lymphocytic infiltration around the blood vessels of the lumbar swelling, including the meninges, in mouse 5. Gallocyanin. × 115.

(*e*) Smith and Quigley stain[8]: This is used in staining for myelin sheath.

1. Decerate and hydrate.
2. Place slides in mordant (*l*) for fifteen minutes.

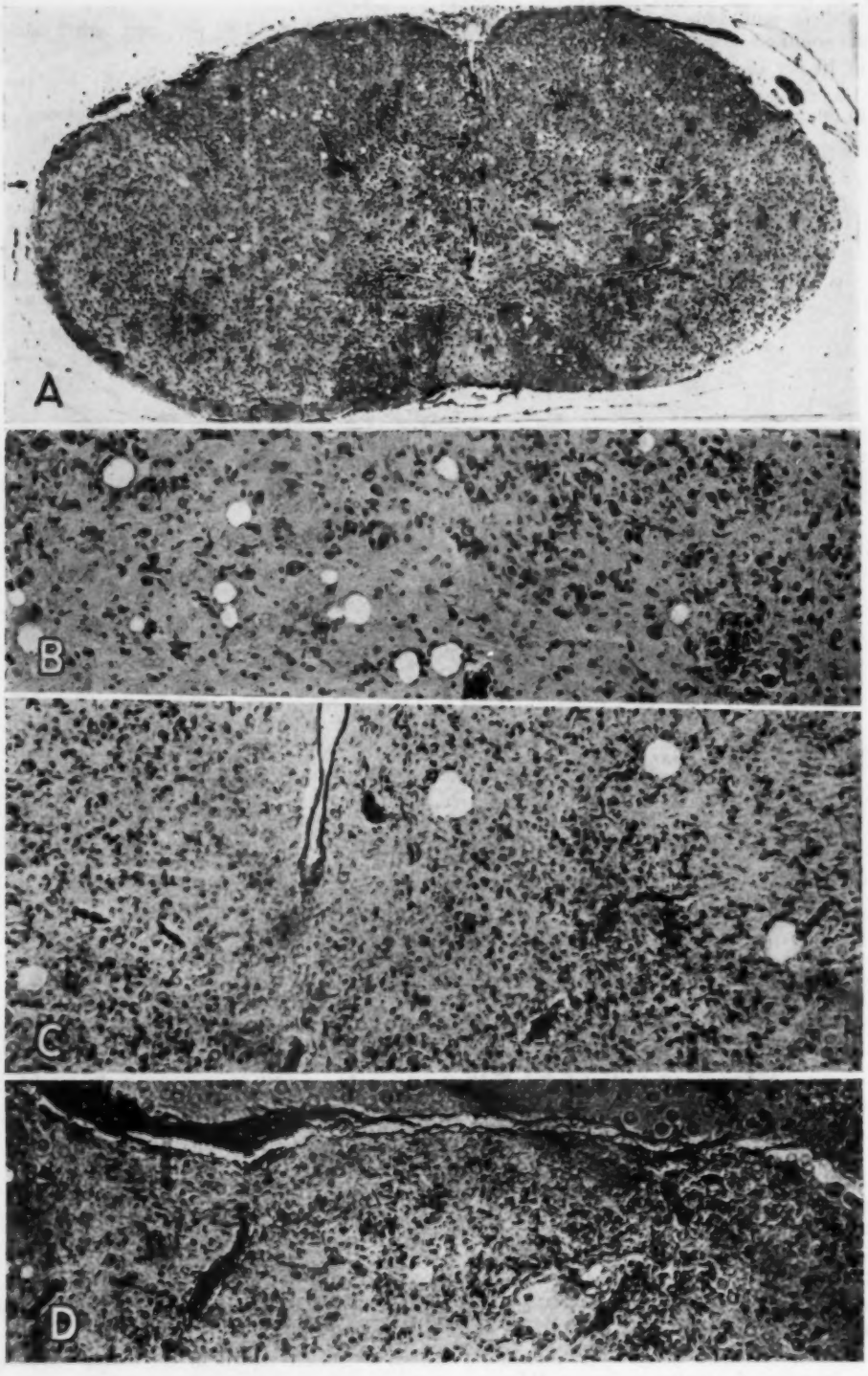

Fig. 4.—*A*, cross section through the medulla oblongata of mouse 2. Note the absence of ganglion cells, the glial proliferation and the lymphocytic infiltrations. Azure II-eosin. × 85.

B, section through the posterior portion of the pons of mouse 2 showing glial proliferation and lymphocytic infiltrations. Azure II-eosin. × 138.

C, glial proliferation and perivascular lymphocytic infiltration around the aquaeductus Sylvii in the midbrain region of mouse 2. Azure II-eosin. × 103.

D, area of injection in the midthalamic region of mouse 2. Azure II-eosin. × 103.

3. Dip slides in 70 per cent ethyl alcohol. Gently raise and lower in alcohol until this runs off in sheets instead of streaks.
4. Place in Smith-Quigley hematoxylin (*m*) at 37 C. for time sufficient to give desired effect (one to two minutes).
5. Dip in single-distilled water as described in step 3.
6. Repeat step 5 in another vessel.
7. Dip in 10 per cent ethyl alcohol as described in step 3.
8. Dip in 30 per cent ethyl alcohol as described in step 3.
9. Dip in 50 per cent ethyl alcohol as described in step 3.
10. Dip in 70 per cent ethyl alcohol as described in step 3.
11. Dip in 83 per cent ethyl alcohol as described in step 3.
12. Dehydrate, clear and mount.

RESULTS

Some results obtained with the method described here are presented in figures 2 to 4. The mice whose tissues are shown in these pictures became paralyzed seven to nine days after inoculation and were killed on the day paralysis developed.

Figures 2 *A* to 3 *C* were taken from sections through the lumbar swelling of the cord. Figures 2 *A* to 3 *B* represent sections of the anterior horns; figure 3 *C*, a section of one half of the cord. The sections shown in figure 2 were stained with azure II-eosin; those in figure 3 *A* and *C*, with gallocyanin, and those in figure 3 *B*, with Bodian's stain. Figure 2 *A* shows coagulation necrosis of ganglion cells and figure 2 *B* varying degrees of loss of staining power up to complete fading out of the cells as described by Wolf.[1e] Figures 2 *C* and 3 *A* present invasion of necrotic ganglion cells by leukocytes and macrophages, while figure 3 *B* shows that the nerve fibers in these areas are apparently normal. Figure 3 *C*, finally, reveals loss of ganglion cells, glial proliferation and lymphocytic infiltration around the blood vessels of the lumbar swelling, including the meninges.

Figure 4 *A* is a good illustration of loss of ganglion cells, glial proliferation and perivascular lymphocytic infiltration in the medulla oblongata, while figure 4 *B* shows similar changes in the pons, and figure 4 *C*, around the aquaeductus Sylvii. Figure 4 *D* shows severe necrosis, vacuolar degeneration and other changes in the midthalamic region, where the injection was given.

This report is not concerned with a discussion of the significance of the lesions. Nevertheless, it may be stated that in the brains and cords studied here the pathologic picture was essentially the same as that reported by Lillie and Armstrong,[1a] Jungeblut and Sanders[1b] and Wolf,[1e] and it compares well with that of poliomyelitis in other animals and in man.

SUMMARY

A method for the histologic study of the central nervous system of the mouse, which has been developed in the Children's Hospital of Philadelphia during a course of studies of the relation of diet to resistance to the Lansing strain of poliomyelitis virus in this animal, clearly demonstrates the alterations of brain and spinal cord characterizing infection with this virus.

STUDIES ON INFLAMMATION

VI. INHIBITORY ACTION OF TUBERCULIN ON CATHEPSIN

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In spite of half a century of intensive research, the mechanism of the tuberculin reaction is still unsolved. On the basis of tissue culture studies, Moen [1] concluded that tuberculin allergy is not the result of an antigen-antibody reaction, that "sensitivity to tuberculin is an inherent characteristic of cells from tuberculous animals" and that "the nature of the toxic action of tuberculin on cells from tuberculous animals when tested *in vitro* is still a matter of conjecture."

While investigating the cytotoxic action of tuberculin on splenic explants in tissue culture, Moen made the significant observation that cells taken from animals which had been infected with a virulent strain (H 37) of Mycobacterium tuberculosis were less active in their initial growth than were those removed from animals infected with an avirulent culture (R_1). There was a close correlation between the pathologic changes in spleens and the growth capacity of these explants in normal culture mediums. These results are strikingly parallel to data which we recently obtained in the course of a study of the kinetics of cathepsin II, prepared from the organs of experimental animals.[2] We showed that the spleens of animals infected with tubercle bacilli by the intravenous route suffer a significant decrease in the speed of their catheptic activity, the reduction being greater when virulent bovine (Ravenel) bacilli are injected than when a lowly virulent (R_1) strain is employed.

In view of these interesting results, additional enzymologic studies were undertaken with the object of finding in tuberculin a catheptic inhibitor substance, which might contribute to its cytotoxic action. This approach seemed indicated since cathepsin is an endocellular proteolytic enzyme presumably concerned in the processes of cellular growth, repair and inflammation.[3]

From the Research Laboratories, Mount Zion Hospital.

1. Moen, J. K., and Swift, H. F.: J. Exper. Med. **64**:339, 1936.

2. Weiss, C., and Halliday, N.: Federation Proc. **2**:96, 1943.

3. Goldshteyn, B., and Tsiperovich, A.: Biochem. J. (Ukraine) **11**:53, 1938; abstracted, Chem. Abstr. **32**: 6679, 1938. Bromley, N. W., and Orechewitsch, W. N.: Biochem. Ztschr. **272**:324, 1934. Anson, M. L.: J. Gen. Physiol. **23**:695, 1940.

4. Lurie, M. B.: J. Exper. Med. **57**:181, 1933.

EXPERIMENTAL PROCEDURE

Inoculation of Animals.—Following the methods of Lurie,[4] rabbits were immunized by two or three monthly intravenous injections of 1 mg. of a nonvirulent strain (R_1) of M. tuberculosis. From this group animals were selected which had become reactive to tuberculin. They were given intravenously several months later 0.1 mg. of a virulent bovine strain (Ravenel) and were killed two weeks after reinfection, since at this time Lurie observed the most pronounced differences between normal and immunized animals (series D). A second group of animals were similarly sensitized with an R_1 culture and tested with tuberculin but were not reinfected (series B). A third group of normal rabbits were each given intravenously 0.1 mg. of the virulent Ravenel strain and killed two weeks later (series C). A fourth group of normal rabbits (series A) was used as a control. Each group comprised 15 to 18 animals. They were exsanguinated from the carotid artery and killed by a blow on the head. The liver, the spleen and the kidneys were removed from each rabbit and frozen immediately in a dry ice (solid carbon dioxide) refrigerator at —70 C. In each group organs from the animals were pooled for preparation of enzyme extracts. In the case of normal spleens it was found necessary, because of their small size, to obtain additional material from an animal dealer. This was kept frozen until delivered in the laboratory.

Pathologic Studies.—Histologic examinations confirmed Lurie's findings.[4] The animals which were infected for the first time with a virulent bovine (Ravenel) culture of tubercle bacilli showed in their livers and spleens numerous tubercles and occasionally giant cells. Intact tubercle bacilli and acid-fast particles were found within epithelioid cells, and the first stages of caseation had appeared in some of the tubercles. In the lungs and the kidneys the lesions were rapidly advancing. There were early tubercles and young epithelioid cells in the lymph follicles. In addition there was an extensive diffuse infiltration of mononuclears in the septums with numerous mitoses, and the first stages of caseation had appeared in the larger tuberculous foci.

The animals which were reinfected with a Ravenel strain after immunization with nonvirulent cultures showed in their livers and spleens an occasional Langhans giant cell or a collection of disintegrating epithelioid cells. In the kidneys no tubercles were present. In the lungs the tubercles had resolved. The cytoplasm of the epithelioid cells was filled with vacuoles, which in some coalesced, leaving only shreds of cytoplasm.

Preparation of Enzyme of Solutions.—The enzyme solutions (cathepsin II) were prepared from the animal

tissues, as previously described, according to the method of Fruton and Bergmann.[5]

In preliminary experiments we tested the inhibitory action of old tuberculin, employing Long's synthetic medium as a control. Both of these materials, however, were so highly colored that the titration end points[6] could not be determined with any degree of accuracy. From Dr. Florence Seibert, of the Phipps Institute, we obtained a sample of a purified protein derivative of tuberculin (PPD)[7] which she describes as follows: "Highly purified and very potent PPD which I prepared in 1940, using ice box temperature throughout and no preservative except a little toluol. It is dry and in vacuo, in the lyophilized form." For the experimental work the material was dissolved in distilled water and dilutions were made so that the final concentrations of purified protein derivative in various experiments ranged from 1×10^{-1} to 1×10^{-7}. These solutions were prepared at frequent intervals.

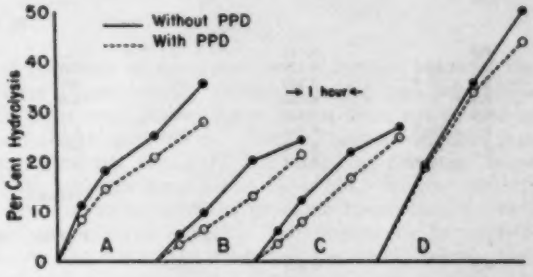
Because of the instability of the dialyzed enzyme[2] it was necessary to study its activity with and without purified protein derivative simultaneously. In some of the hydrolysis tubes, therefore, enzyme (at several levels of protein nitrogen), cysteine-buffer, solution of purified protein derivative and the required amount of water were incubated at 40 C. for two hours before the substrate benzoyl-l-arginineamide (BAA) was added and measurement of hydrolysis begun. In another set the mixture of the substrate, cysteine-buffer solution and water was warmed to 40 C., the desired amount of enzyme added, and titrations started immediately. All samples were chilled and titrated immediately after digestion. The increases in titer at time t over that at zero minutes were taken as the measure of hydrolysis. According to the method of Grassmann and Heyde,[6] a difference of 1 cc. of one-hundredth molar alcoholic potassium hydroxide represents 100 per cent splitting of a peptide bond.

RESULTS

As shown in the accompanying figure, purified protein derivative caused approximately the same decrease in the speed of activity of cathepsin II whether the enzyme was obtained from the spleens of normal animals or from those of animals infected with virulent or with nonvirulent tubercle bacilli. The reduction was approximately 25 per cent. Moreover, there were no differences in the effects exerted on splenic cathepsin by concentrations of purified protein derivative varying from 1×10^{-1} to 1×10^{-7} mg. per cubic centimeter of test solution and none when we used the same concentration of purified protein derivative with different levels of enzyme. The inhibition was usually greater at the three hour interval than earlier, but in a few experiments carried out at room temperature or in the ice box, so as to slow down enzyme activity, the results after longer intervals of time were essentially the same. Since the curves of activity flattened after three hours, data beyond this time were affected by this factor. These results show a similarity to those of Moen on splenic explants grown in tissue culture, since he too observed that old tuberculin inhibits equally cells removed from animals infected with virulent or with nonvirulent tubercle bacilli. For purposes of comparison we included liver and kidney, organs which were not examined by Moen. The results (table) indicate that purified protein derivative is a general inhibitor of catheptic enzyme activity and exerts no selective action, the degree of inhibition showing no relation to the immunologic source of the material.

COMMENT

This investigation was begun with the hope of throwing light on the mechanism of the cytotoxic action of tuberculin in vitro. Our previous enzymologic data showed that the decreases in the speed of activity of splenic cathepsin were parallel to Moen's observations on the reduction



The curves show the per cent hydrolysis (in the presence and the absence of purified protein derivative of tuberculin [*PPD*]) of benzoyl-l-arginineamide by cathepsin II prepared from spleens of normal rabbits (series A), those infected with an avirulent (R_A) strain of M. tuberculosis (series B), a virulent (Ravenel) strain (series C) and animals given an injection of R_A and reinfected with the Ravenel strain (series D).

The concentrations of enzyme, expressed as milligrams of protein nitrogen per cubic centimeter of test solution, and of purified protein derivative, expressed as milligrams per cubic centimeter of test solution, in series A, B, C and D, respectively, were as follows: (*A*) enzyme 0.072 mg., purified protein derivative 3.2×10^{-2} mg.; (*B*) enzyme 0.060 mg., purified protein derivative 3.2×10^{-2} mg.; (*C*) enzyme 0.064 mg., purified protein derivative 1×10^{-2} mg.; (*D*) enzyme 0.092 mg., purified protein derivative 3.2×10^{-2} mg.

in the speed of growth of splenic explants removed from animals infected with virulent or with nonvirulent tubercle bacilli. In the present communication we have demonstrated another parallelism with Moen's results: Purified protein derivative inhibits to an equal degree catheptic enzymes derived from spleens of animals infected with either virulent or nonvirulent tubercle bacilli just as tuberculin inhibits equally the growth of splenic explants derived from

5. Fruton, J. S., and Bergmann, H.: J. Biol. Chem. **130**:19, 1939.

6. Grassmann, W., and Heyde, W.: Ztschr. f. physiol. Chem. **182**:32, 1929.

7. Seibert, F. B., and Glenn, J. T.: Am. Rev. Tuberc. **44**:9, 1941.

similar sources. Moreover, purified protein derivative apparently acts as a general inhibitor of cathepsin, since it affects similarly enzyme solutions prepared from other organs, such as the rabbit's liver and kidneys. There is no correlation between the virulence of infection (and hence the extent of injury of tissue) and the degree of enzymic inhibition exerted by purified protein derivative in vitro, just as there is no proportionality between the initial growth energy of explants and the degree of inhibition of growth exerted on them by old tuberculin. Our experiments, like those of Moen, have therefore thrown no new light on the mechanism of the cytotoxic action of tuberculin in vitro or on the unsolved problem of tuberculin hypersensitiveness in general.[8]

However, we have discovered that each of two substances, the protein (PPD) and phosphatide [9] fractions, derived from the tubercle bacillus, inhibits endocellular proteolytic enzymes.[10] In view of the work of Jobling and Petersen [11] it is possible that these may be the inhibitors which prevent caseous tuberculous tissue from undergoing autolysis. Since caseation and softening are usually referred to the allergic state of infected animals,[12] it is hoped that further studies along these lines may ultimately throw light on this difficult problem.

Kinetics of the Hydrolysis of Benzoyl-l-Arginineamide (BAA) by Cathepsin II Prepared from Livers of Normal Rabbits and Those of Rabbits Infected With Virulent (Ravenel) or With Nonvirulent (R_1) Tubercle Bacilli. Enzymes Tested With and Without the Presence of Purified Protein Derivative

Concentration of PPD, Mg. per Cc. of Test Solution	Concentration of Enzyme, Mg. of Protein Nitrogen per Cc. of Test Solution	K × 10⁴ *		C × 10² *		Unit *		Unit per Mg. of Protein Nitrogen per Cc. of Test Solution	
		Without PPD	With PPD	Without PPD	With PPD	Without PPD	With PPD	Without PPD	With PPD
Normal (Series A)									
3.2 × 10⁻²	0.095	6.9	4.8	7.3	5.1	0.26	0.40	3.6	2.5
3.2 × 10⁻³	0.180	14.0	8.2	7.4	4.3	0.28	0.46	3.7	2.2
1.28 × 10⁻³	0.157	10.8	6.8	6.9	4.3	0.29	0.46	3.5	2.2
3.1 × 10⁻³	0.157	....	9.9	...	6.3	....	0.32	...	3.2
6.4 × 10⁻³	0.157	....	9.2	...	5.9	....	0.34	...	3.0
1.3 × 10⁻¹	0.157	....	10.6	...	6.8	....	0.30	...	3.4
			Average:	7.2	5.5	0.28	0.38	3.6	2.8
R1 (Series B)									
0.0	0.047	2.7	...	5.7	...	0.35	....	2.9	...
3.2 × 10⁻²	0.118	6.7	5.6	5.7	4.8	0.35	0.42	2.8	2.4
3.2 × 10⁻³	0.180	10.7	9.2	5.7	4.9	0.35	0.41	2.8	2.5
3.2 × 10⁻³	0.354	19.0	16.0	5.3	4.5	0.38	0.45	2.7	2.3
0.0	0.440	27.6	...	6.3	...	0.32	....	3.1	...
1 × 10⁻¹	0.164	12.3	7.1	7.5	4.3	0.27	0.47	3.7	2.2
1 × 10⁻³	0.164	....	9.2	...	5.6	....	0.36	...	2.8
1 × 10⁻⁵	0.164	....	9.5	...	5.8	....	0.34	...	2.9
			Average:	6.0	5.0	0.34	0.41	3.0	2.5
Primary Ravenel (Series C)									
0.0	0.178	13.3	...	7.5	...	0.27	....	3.8	...
0.0	0.232	15.4	...	6.6	...	0.30	....	3.3	...
0.0	0.237	16.8	...	7.1	...	0.28	....	3.5	...
1 × 10⁻¹	0.116	10.3	8.9	8.7	7.7	0.23	0.36	4.3	3.8
1 × 10⁻²	0.116	....	8.5	...	7.3	....	0.27	...	3.7
1 × 10⁻⁴	0.116	....	8.9	...	7.6	....	0.26	...	3.8
1 × 10⁻⁶	0.116	....	7.1	...	6.2	....	0.33	...	3.1
			Average:	7.5	7.2	0.27	0.28	3.7	3.6
Reinfection with Ravenel (Series D)									
3.2 × 10⁻²	0.082	7.9	3.9	9.7	4.7	0.21	0.43	4.8	2.3
3.2 × 10⁻³	0.164	10.8	8.7	6.6	5.3	0.30	0.38	3.3	2.7
0.0	0.197	12.0	...	6.1	...	0.33	....	3.0	...
1.28 × 10⁻⁴	0.174	9.4	8.2	5.4	4.7	0.37	0.42	2.7	2.4
6.4 × 10⁻³	0.174	....	10.8	...	6.2	....	0.32	...	3.1
3.1 × 10⁻³	0.174	....	9.2	...	5.3	....	0.38	...	2.7
1.3 × 10⁻¹	0.174	....	9.7	...	5.6	....	0.36	...	2.8
			Average:	7.0	5.3	0.30	0.38	3.5	2.7

* K, C and unit values represent the average of individual results for each interval of time from one to three hours.

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ROLE OF ACETYLCHOLINE IN THE ANAPHYLACTIC PROCESS

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It has been demonstrated that histamine-like substances are released into the blood stream and from the tissues, particularly the lungs, during anaphylactic shock.[1] The pharmacologic action of histamine, however, does not duplicate all of the manifestations of anaphylactic shock.[2] The role of potassium has been discussed.[3] That acetylcholine might play some part in the production of the symptoms of anaphylactic shock has been suggested on theoretic grounds, and this suggestion has been subjected to experimental evaluation with conflicting results. Wenner and Buhrmester [4] found that potassium and a substance resembling acetylcholine were released into the blood stream after the administration of shock doses of antigen in sensitized rabbits. Ratnoff,[5] with the aid of technics carefully considered to exclude experimental error in testing for acetylcholine, found no direct evidence on the physostigminized leech muscle preparation to support the view that acetylcholine was released into the blood stream of rabbits subjected to anaphylactic shock. Went and Lissak [6] reported that choline appeared in the perfusate from the guinea pig heart in anaphylactic shock. He concluded from a series of studies that at least three different shock substances—histamine, choline and acetylcholine—may be released from different organs in the course of the anaphylactic process.

Anaphylactic shock appears [7] to be independent of the direct action of nerves. There is marked similarity, however, of the effects on the respiratory tract of the guinea pig produced by acetylcholine, electrical stimulation of the vagus nerves and anaphylactic shock.[7] Bronchoconstriction of severe degree is produced in all three instances. Since acetylcholine is liberated in the lungs immediately after stimulation of the vagus nerves in the guinea pig,[8] it appeared worth while to attempt by technics different from those previously employed to ascertain whether acetylcholine is released in anaphylactic shock in the guinea pig. It was decided also to utilize the material available in this study for further examination of the hypothesis that histamine is involved in anaphylactic shock.

METHODS

As sources of sensitized tissues for our study of the effects of anaphylactic shock we used guinea pigs which had been sensitized to horse serum from twenty-one to forty days previously. Similar normal guinea pigs that were not sensitized were used as controls. Most of the animals were killed by a blow on the occiput; a few, by an injection of procaine hydrochloride made into the cisterna magna. The organs to be studied, chiefly the heart, the lung and the small bowel, were removed quickly, divided into two portions each and washed, one in Tyrode's solution, the other in Ringer's solution without added physostigmine (later referred to as plain Ringer's solution). Sections of bowel were always first spread longitudinally along the mesenteric attachment and the fecal material gently wiped of the mucosa before they were placed in the wash solutions. The two portions of organ were allowed to wash in these solutions for ten to fifteen minutes, until relatively free of blood. They were then ready for assay.

One portion of each organ, that placed in plain Ringer's solution, was assayed for acetylcholine after having been shocked in vitro by horse serum according to an adaptation of the method of Schild [9] as described later in this paragraph. For the assay of acetylcholine the following convenient method was used: The dorsal body muscle of a leech was divided longitudinally into two equal portions, each of which was then suspended in an 8 cc. bath containing 5 cc. of Ringer's solution through which a small stream of air was bubbled. The free end of each muscle was attached to a writing lever which recorded the length of the muscle on a smoked drum. One muscle was suspended in plain Ringer solution; the other, in Ringer solution to which had been added sufficient physostigmine salicylate to result in a concentration of the latter of 1 part in either 250,000 or 500,000. The Ringer solution used was similar to

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From the departments of pathology of Harvard Medical School, the Infants' Hospital and the Children's Hospital.

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that previously employed.[8] The solution was used at room temperature and adjusted to p_H 6.9. The leech muscles were allowed to remain in their aerated baths for at least thirty minutes and before use for assays of acetylcholine were treated always with acetylcholine in a concentration of 1×10^{-8}, to determine whether the preparation would give the reaction characteristic of the effect of acetylcholine (contraction in the presence of physostigmine but no reaction in the absence of this agent). The organs to be assayed, after being removed and washed as previously described, were divided into small pieces: each pulmonary lobe into two or three fragments, the heart into six to eight portions and the bowel into eight to ten segments, each about 2 cm. in length. In a few cases all of the tissues were as finely divided as possible with small scissors. Half of each group of pieces was placed in from 6 to 12 cc. of plain Ringer's solution and the other half in similar amounts of Ringer's solution with added physostigmine. To each was added from 0.05 to 0.2 cc. of anti-influenza type B horse serum as prepared by the Massachusetts State Antitoxin Laboratory, which had been used to sensitize the guinea pigs employed in this study. The tissues were allowed to stand for periods of time varying from one minute to four hours. The solutions in which they had been standing were then tested on the appropriate leech muscle. The response of the latter, if any, was observed for from three to five minutes. The great majority of the fluids were tested after standing either ten or thirty minutes. A response was considered positive if the physostigminized leech muscle contracted in the manner characteristic of its response to acetylcholine and if there was no similar contraction of the nonphysostigminized leech muscle.

In a number of experiments the effect of the fluids bathing the normal or the sensitized tissues treated with antigen was observed on the blood pressure of cats. Cannulation of the tracheas and carotid arteries of normal cats was done so as to obtain a continuous recording of arterial blood pressure and respirations: 1 cc. amounts of fluids simultaneously assayed on leech muscles were injected into the external jugular veins of the cats and the effects on the blood pressure of the cats were observed. Such observations were made both before and after an intravenous injection of 0.25 mg. of prostigmine bromide and before and after a similar injection of 4.0 mg. of atropine sulfate.

Many control studies were carried out on the tissues of normal guinea pigs treated in exactly the same fashion as the sensitized animals. During each experiment the sensitivity of the physostigminized leech muscle to acetylcholine at a concentration of 1×10^{-8} was checked repeatedly. At the termination of each experiment the control leech muscle was similarly phosostigminized and the sensitivity of both muscles to acetylcholine in the following concentrations determined: 5×10^{-9}, 1×10^{-8} and 1×10^{-7}. All leech muscles gave characteristic responses to acetylcholine in the concentration of 1×10^{-8}. A few responded to the concentration of 5×10^{-9}.

In a number of experiments, Tyrode solution in which lung from a sensitized guinea pig had been shocked in vitro was tested for histamine content on a guinea pig's ileum prepared as follows: A portion of small intestine removed from a guinea pig freshly killed by a blow on the head was placed in Tyrode's solution and kept in a refrigerator until ready for use. When needed a portion of ileum, about 2 cm. long, from which the mesentery had been removed, was washed by allowing Tyrode solution to run through its lumen by gravity. This piece of small bowel was then suspended by means of silk ligatures in a 5 cc. bath through which was bubbled a small stream of oxygen. This 5 cc. bath was immersed in a large water bath maintained at a constant temperature—37 C. The piece of ileum was kept in it for thirty to ninety minutes before use. The preparation was then exposed to a solution of histamine in the concentration of 10^{-7}, which was achieved most simply by adding 0.5 cc. of a solution of histamine in the concentration of 10^{-6} to the 5 cc. bath. A vigorous contraction of this preparation under these conditions is characteristic of histamine. The test was recorded for thirty seconds, at which time the contraction reached its peak. The 5 cc. bath was then washed thoroughly three times with fresh Tyrode solution. After a thirty second pause the preparation was again ready for use.

Since cool or cold solutions or extracts of organs being tested for histamine content may cause contraction of the ileum prepared as described, each solution to be tested was kept in the constant temperature water bath long enough for its temperature to equal that of the fluid surrounding the suspended piece of ileum in the 5 cc. bath. In the experiments reported here all in vitro shocking of the organs tested was carried out in containers suspended in the large water bath at 37 C.

RESULTS

Histamine.—The fluid which bathed pieces of lung shocked in vitro was examined for histamine in 15 instances. In 2 instances no response was obtained. In the remaining ones a substance which exhibited the pharmacologic behavior of histamine was present in amounts calculated to range from 0.01 to 2.0 micrograms per gram of lung tested. Schild[9] obtained an average of 2.23 micrograms of histamine per gram of lung shocked in vitro. He found that his controls (not shocked) either were inactive or produced an insignificant effect.

Acetylcholine.—The results are summarized in table 1. Acetylcholine was demonstrated in both the normal and the shocked lung in a small percentage of trials, and then only when solutions with added physostigmine were used. A large number of experiments were performed with lung specimens because the lung is the "shock organ" of the guinea pig and special effort was used to determine the conditions under which the few positive responses were obtained. Positive results were not obtained with extracts of normal hearts in 27 trials but were noted with 10 per cent of extracts of shocked hearts when physostigmine was present in the extraction solution. The results were much the same with extracts of normal bowels and shocked bowels. When solutions with added physostigmine were employed, there was demonstrated in two thirds of each series the release of a substance having the characteristics of acetylcholine under the conditions of the experiment.

Of the tissues studied, the most marked responses were obtained with bowel tissues from either normal or shocked animals. In the physostigminized extracts there were found concentra-

tions of acetylcholine from 1 : 10,000,000 to 1 : 100,000,000, estimated by comparison with the effects on the same leech muscle of known amounts of acetylcholine chloride. Further proof that the substance liberated was acetylcholine was seen in the effect on the blood pressure of a cat which had received an intravenous injection of 1 cc. of Ringer's solution in which bowel had been standing for thirty minutes. Repeated observations were made in each of 5 cats. There was no effect on the blood pressure when 1 cc. of plain Ringer's solution in which bowel had been standing for thirty minutes was used. When 1 cc. of extract in Ringer's solution with added physostigmine was injected, the blood pressure was lowered an average of 17 mm. of mercury. After injection of prostigmine in a vein of the cat a repetition of the same dose of extract in Ringer's solution with added physostigmine caused a lowering of 22 mm. of mercury. The injection of the same amount of the extract after atropine sulfate had been injected into a vein of the cat caused a lowering of the blood pressure of only 5 mm. of mercury. The lowering of the blood pressure of the cat, intensified by prostigmine and diminished or abolished by atrophine, is characteristic of the action of acetylcholine. The slight lowering of the blood pressure after the injection of atropine sulfate was caused probably by other substances present in the extract of the intestinal tissue.

TABLE 1.—*Determinations of Acetylcholine in Organs of Guinea Pigs*

	Experiments	Responses
Lung		
Normal		
(1) Extracted in plain Ringer's solution	25	0
(2) Extracted in Ringer's solution with added physostigmine	40	3
Shocked		
(1) Extracted in plain Ringer's solution	30	0
(2) Extracted in Ringer's solution with added physostigmine	56	5
Heart		
Normal		
(1) Extracted in plain Ringer's solution	10	0
(2) Extracted in Ringer's solution with added physostigmine	27	0
Shocked		
(1) Extracted in plain Ringer's solution	23	0
(2) Extracted in Ringer's solution with added physostigmine	31	3
Bowel		
Normal		
(1) Extracted in plain Ringer's solution	26	1
(2) Extracted in Ringer's solution with added physostigmine	27	18
Shocked		
(1) Extracted in plain Ringer's solution	24	1
(2) Extracted in Ringer's solution with added physostigmine	25	17

The effect of the length of time during which the tissues were permitted to stand in the solutions employed on the liberation of acetylcholine from the bowel in vitro is shown in table 2. Positive results were noted as early as ten minutes after the start of the experiment, but the best results were obtained after the experiment had been under way for thirty to sixty minutes or longer.

A series of pertinent controls of the studies on the leech preparations were carried out. The horse serum utilized in the shock experiments caused no contraction in the leech muscle in 11 trials. Potassium chloride in concentrations varying from 500 mg. to 5 mg. per hundred cubic centimeters had no effect on the leech preparation which could be confused with that produced by acetylcholine. Relaxation of the preparation was noted occasionally, and sometimes there was an irregular series of contractions and relaxations. The effect of histamine on the leech preparation was observed with 1 cc. amounts of solutions of histamine phosphate at concentrations of 10^{-6}, 10^{-4}, 10^{-5}, 2×10^{-7}, 1×10^{-7} for a total of 11 trials without a positive result.

COMMENT

No evidence was obtained that acetylcholine was released in amounts or in frequency any greater from sensitized guinea pig lungs shocked in vitro than from normal lungs under the conditions of the experiment. Acetylcholine could be demonstrated in 10 per cent of shocked hearts and in none of normal hearts. The percentage of positive responses, while small, suggests the value of further study with methods more delicate than those employed in this study. It is in agreement with the conclusions of Went and Lissak [6] concerning the release of a choline-like substance from the heart in anaphylactic shock. No significance in the anaphylactic process can be attached to the findings of acetylcholine in the physostigminized solution of sensitized guinea pig bowels shocked in vitro since there was just as high a percentage of positive findings with normal bowels when physostigminized solutions were employed. The failures with one third of both the normal and the shocked bowels may be attributed to the short period the tissues were permitted to stand in these experiments with nega-

TABLE 2.—*Effect of Time on Amount of Acetylcholine Liberated from Bowel Subjected to Saline Extraction*

Period of Standing, Minutes	Experiments	Responses	Percentage of Positive Results	Average Magnitude of Responses	Approximate Concentration of Acetylcholine
1	1	0	0		
5	1	0	0		
10	14	5	36	+	1×10^{-6}
15	1	0	0		
30	24	22	92	++	1×10^{-6}
45	2	1	50		
60	5	5	100	++++	1×10^{-7}
Over 60	4	4	100	++++	1×10^{-7}

tive results. Of considerable interest was the demonstration that acetylcholine could be liberated so easily from decomposing intestinal tissue when physostigmine was used to inhibit the hydrolysis of acetylcholine. The method of extraction employed in these studies appears to have value in studies concerning the liberation of acetylcholine from the other tissues of the body.

Our studies confirm the repeated demonstration that histamine is liberated from tissues subjected to anaphylactic shock.

SUMMARY

In experiments designed to test the possibility that acetylcholine might be liberated in the anaphylactic process in the guinea pig, saline extracts of cardiac, pulmonary and intestinal tissues of normal and of sensitized guinea pigs, prepared with and without physostigmine, were examined for acetylcholine content. Pieces of the organs of the sensitized guinea pigs were shocked in vitro according to the method of Schild.[9] The number of positive responses and the amount of acetylcholine liberated were no greater with the shocked pieces of lungs and intestines than with the normal controls. Histamine was demonstrated with regularity in the shocked pieces of lungs. In 10 per cent of the extracts of shocked heart and in none of the extracts of normal heart acetylcholine was demonstrated. The ease of demonstrating the formation and liberation of acetylcholine from the intestine of the normal guinea pig by means of this simple saline extraction technic is emphasized. The amount liberated under these conditions varies directly with the duration of the saline extraction.

Mr. John Carabitses gave valuable technical assistance.

FATE OF POLYVINYL ALCOHOL INTRODUCED INTRAPERITONEALLY IN RATS

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Hueper [1] reported the pathologic effects of polyvinyl alcohol after it has been injected subcutaneously and intravenously in rats and rabbits respectively. This was the first report on pathologic effects of polyvinyl alcohol. An earlier paper by Herrmann and Haehnel [2] had indicated no untoward effects, but the conclusions were based on meager data. No other reports on biologic effects appeared in the interim.

Hueper demonstrated that while many tissues remained normal after the injection of polyvinyl alcohol, others became depots for small to large amounts of the substance. Particularly was it found in the kidneys in large amounts. The endothelial cells of the kidneys, the lungs and the brain, the reticuloendothelial cells of the spleen, the liver and the lymph nodes, the histiocytes of various organs and occasionally the glia and the ganglion cells of the brain absorbed the material.

Hueper, Landsberg and Eskridge [3] reported the effects of intravenous and intraperitoneal injections of polyvinyl alcohol on the blood of dogs and rabbits. A single injection caused an immediate decrease in erythrocytes. Repeated injections produced progressive anemia. The results on the blood and the tissues were similar to those produced by solutions of acacia. A 5 per cent solution was used in all of Hueper's work, and the dosage ranged from about 100 to 1,000 mg. per kilogram.

Young, Mulay and Christie [4] reported the effects of single massive doses (1 Gm. per kilogram) of polyvinyl alcohol on the renal tissue and the blood of dogs. Immediate changes in the blood occurred, but all values returned to normal within one to three weeks after the injection except the sedimentation rate, which remained faster than normal for more than six weeks. Polyvinyl alcohol could be traced in the blood for as long as fifty days. They found it accumulating in the liver, the spleen and the kidneys. They concluded that it is probably dangerous as a therapeutic agent.

EXPERIMENTS

Rats and guinea pigs were given intraperitoneal injections of polyvinyl alcohol. In guinea pigs no appreciable accumulation in either the liver or the kidneys was demonstrated. Rats were therefore employed for the main experiments. The evaluation of the pathologic changes in these experiments is based on the renal area involved by the injected substance. The typical hyalin-like deposit in the capillary tufts and elsewhere was described and illustrated by Hueper. Our lesions appeared identical with those in his figure 5 except for varying degree. We have indicated the amount of glomerular involvement by plus signs.

TABLE 1.—*Development of Lesions in the Kidneys of Rats After Injection of Polyvinyl Alcohol*

Rat	Body Weight, Gm.		Time Elapsed, Weeks	Dose, Mg. per Kg.*	Degree of Glomerular Involvement †	
	Initial	Final			Hematoxylin and Eosin	Iodine
101	134	172	1	10 A	+	
102	139	219	3	10 A	0	
103	133	342	8	10 A	0	0
104	130	156	1	10 B	0	
105	129	228	3	10 B	0	0
106	150	274	8	10 B	0	
107	114	162	1	50 A	0	
108	122	211	3	50 A	±	0
109	122	223	8	50 A	±	±
110	135	177	1	50 B	+	
111	121	219	3	50 B	±	±
112	110	279	8	50 B	0	0
113	129	170	1	250 A	±	
114	122	197	3	250 A	++	+
115	144	327	8	250 A	++	++
116	132	160	1	250 B	+	
117	131	231	3	250 B	+	±
118	119	261	8	250 B	+	+
119	141	173	1	1,000 A	±	
120	126	211	3	1,000 A	±	±
121	128	266	8	1,000 A	+++	+++
122	151	194	1	1,000 B	±	
123	125	232	3	1,000 B	++	++
124	119	292	8	1,000 B	+++	++

* In this column A signifies lot 128 of polyvinyl alcohol and B, lot 149 of RH 391.

† In this column 0 means that no deposit of polyvinyl alcohol was observed; ± indicates that a trace or a slight amount was seen; +, 25 per cent; ++, 50 per cent, and +++ indicates that there was involvement of 75 per cent of the glomerular area, respectively.

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The results shown in table 1 are based on single intraperitoneal doses ranging from 10 to 1,000 mg. per kilogram of polyvinyl alcohol (du Pont). The rats were killed at one, three and eight weeks after the injection. Sections of liver and kidneys were obtained and stained with hematoxylin and eosin and with iodine following Hueper's technic.[1] Polyvinyl alcohol was not found in the liver except in 1 rat receiving a dose of 1,000 mg. per kilogram, and then only a trace. Data on the liver are omitted since they were nearly all negative. The results in control animals were likewise negative. Quantitative differences between the results with iodine and with hematoxylin and eosin are probably due to chance variation in the distribution of the deposits.

TABLE 2.—*Deposition and Elimination of Polyvinyl Alcohol After Single Injections of About 500 Mg. per Kilogram*

Rat	Body Weight, Gm. Initial	Body Weight, Gm. Final	Time Elapsed, Months	Degree of Glomerular Involvement: Hematoxylin and Eosin	Degree of Glomerular Involvement: Iodine
39	207	223	1	+	+
41	192	217	1	+	++
43	160	221	2	+++	+++
45	168	207	2	++	++
47	171	225	4	+++	++
49	210	263	4	+++	++
51	175	374	6	+++	+++
53	192	370	6	+++	++
48	209	368	7 (died)	± to +	
52	175	138	8½ (died)	+	+
40	186	346	12	++	+
54	198	320	11 (died)	++	++
55	222	394	12 (died)	± to +	±
42	185	406	15	±	± to +
44	213	465	15	0	0
38	207	346 *	19 (died)	±	+
50	197	320 †	19½ (died)	± to +	+

* At fifteen months the maximum weight was 444 Gm.
† At fifteen months the maximum weight was 476 Gm.

The dose of 10 mg. per kilogram gave practically negative results. Doses of 50, 250 and 1,000 mg. per kilogram produced progressively greater deposits of polyvinyl alcohol; also the longer the elapsed time, the greater the deposit. Two separate lots of polyvinyl alcohol, labeled A and B in table 1, gave substantially identical results.

In an earlier, preliminary experiment 5 rats received 100 mg. per kilogram and were killed one day, one, two, four and six weeks later. The degree of renal involvement as judged by iodine staining was 0, +, ±, ± and 0 for the 5 animals, respectively. This suggested gradual elimination of the deposited alcohol; hence the more extensive schedules shown in tables 1 and 2 were followed.

In table 2 an experiment of longer duration is reported. Single doses of 2 cc. of 5 per cent polyvinyl alcohol were introduced intraperitoneally in each rat (equivalent to 450 to 625 mg. per kilogram, depending on the weight of the rat). The rats were killed at regular intervals except for 6 that died spontaneously. In the untreated control group 2 rats died spontaneously at thirteen and sixteen months whereas 2 treated animals survived as long as nineteen and nineteen and one-half months.

COMMENT

During all the experiments there was no evident loss of appetite. Both the treated and the untreated control animals gained weight uniformly and in a parallel manner, as indicated by the data in table 3. Two of 6 normal animals died spontaneously, and 6 of 17 treated animals died, 2 of the latter surviving longer than the 2 normal animals. The average survival time of the 6 treated ones was thirteen months. The difference is not significant. While the experiment was not designed to evaluate survival times, the results suggest that under the experimental conditions there is no shortening of life expectancy even though renal function can hardly be expected to be other than considerably diminished.

The results show that some time is required to absorb the material from the abdominal cavity and deposit it in the glomeruli. They further indicate that the material is eliminated after some months. At seven to twelve months the lesions are distinctly smaller than in the two to six month period. Animals killed or dying at fifteen months or thereafter showed not only still smaller glomerular lesions but involved areas that appeared normal. This may represent recovery of the involved capillaries or it may represent actual regeneration of capillary endothelium.

TABLE 3.—*Average Weights of Rats Listed in Table 2 and of Control Rats (Not Shown in Table 2)*

Time Elapsed Since Start of Experiment, Mo.	Average Weight of Controls, Gm.	Number	Average Weight of Treated Rats, Gm.	Number
0	205	6	205	17
1	235	6	230	17
2	271	6	256	15
4	326	5	305	13
6	372	4	369	11
9	399	3	407	7
12	402	3	431	6
15	434	2	447	4
16½	...	..	440	2
19 (at death)	...	..	333	2

No one has reported any evident chemical reaction of polyvinyl alcohol in tissues, and Hueper attributed all the effects to physiochemical factors. On consideration of these factors together with

the present data it seems that polyvinyl alcohol might possess clinical usefulness in selected cases. Intravenous injection of extremely small amounts would probably not be dangerous but should be avoided. Intravenous injection of larger doses, as in replacement of plasma, is definitely contraindicated. The use of this alcohol in 1 or 2 cc. doses as a vehicle for intramuscular or subcutaneous injection may not be desirable though it probably would not be dangerous. For intrauterine medication and for oral use the substance would seem to have reasonable safety. While the small number of animals used gives results suggestive rather than conclusive, it hardly seems likely that random distribution of tolerant and susceptible rats would be such as to give these results were gradual elimination and recovery not a fact.

SUMMARY

Polyvinyl alcohol after being intraperitoneally injected in rats is slowly deposited in the kidneys and slowly eliminated. Small doses are completely eliminated in a few weeks. Large doses require a longer time for maximum deposition and a longer time for elimination.

Neither appetite nor growth seems to be affected by massive doses of 0.5 to 1.0 Gm. per kilogram introduced intraperitoneally.

There was a decided difference in reaction between rats and guinea pigs, the latter showing little evidence of deposition of polyvinyl alcohol in the kidneys or the liver.

Case Reports

OSTEOCHONDROMA OF THE BASE OF THE SKULL

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Intracranial cartilaginous tumors are rare. From scattered case reports, four distinct groups can be separated. First are those which arise in the paranasal sinuses and extend only secondarily into the cranial cavity.[1] In a second group may be placed those cartilaginous tumors which are in intimate relation to the meninges.[2] Wolf and Echlin [2b] pointed out that such tumors are not necessarily allied to meningioma and that it is impossible to be certain of the histogenesis of all of them. But it would appear that for the majority of those reported the generic relation to meningioma is probable.

A third group is more poorly defined and more difficult to understand. In this group may be placed the cartilaginous tumors of the brain without evidence of gross attachment to surrounding structures. The tumors reported by Chorobski and co-workers [3] and deBusscher [4] fall into this category. Chorobski and associates suggested that such tumors arise from the arachnoid, although on examination the point of attachment may be overlooked.

The fourth group is clearcut and consists of chondromatous or osteochondromatous growths arising from the base of the skull. Hirschfeld [5] in 1851 was the first to describe such a tumor. It occurred in a woman about 30 years of age, on whom no history was available. Hirschfeld cited Müller, who had collected 34 cartilage tumors, one of which was intracranial. But since no adequate data are given, this tumor in Müller's collection, although possibly of the same type, cannot be unequivocally accepted. No other tumor of this type could be found recorded in the literature until in the last ten years, when reports were published by Levitt,[6] Paleari [7] and Green and Childrey.[8] List [9] collected 5 more tumors of this type, including 2 observed by Cushing. In 4 instances osteochondroma of the sphenoid region was the primary lesion. In the fifth the patient suffered from generalized chondromatosis, and the sphenoid lesion was an intracranial manifestation of the systemic disease. He cited from the literature 2 other possibly similar cases.

The present case is one of primary solitary intracranial osteochondroma arising from the base of the skull. Of the solitary lesions, it is the ninth to be reported in the literature.

REPORT OF A CASE

A 25 year old white man gave a history of "stomach trouble" beginning five years before his entry into the hospital. The symptoms were burning and vague epigastric pain, relieved by sodium bicarbonate and a bland diet. At the time of his induction into the Army in July 1941 he was free of symptoms. In October 1941 he had an attack of vomiting, with coffee ground vomitus, and there was blood in the stools. No changes were demonstrable by roentgenogram. His symptoms cleared rapidly on a bland diet, and he remained well until November 1942, when gastric distress returned. When he was transferred to this hospital, Dec. 12, 1942, a gastrointestinal series of roentgenograms revealed a nonobstructive duodenal ulcer.

Under appropriate treatment his course was uneventful until late in December 1942, when he complained of vague headaches, principally frontal. The eyes, the ears, the nose and the throat were essentially normal. The optic disks were normal and well outlined except for slight blurring at the top of the left disk; there was no elevation; the retinal veins were full, with mild tortuosity; no hemorrhages were present. The visual fields were bilaterally and symmetrically constricted on one examination, but a subsequent examination showed essentially normal fields. Since, in addition, the sinuses were roentgenologically normal, the patient was given clearance by the eye, ear, nose and throat section of the hospital.

However, in the course of the roentgen examination of the skull an unusual mass was noted (fig. 1). It was described as "a large irregular mass of ossified tissue, about 3 by 5 cm. in size, extending from the right lateral margin of the sella turcica in a superior direction. The densities are annular, and some of them appear to follow a tortuous vascular distribution. The

From the Laboratory Section of the William Beaumont General Hospital, El Paso, Texas.

1. Menne, F. R., and Frank, W. W.: Arch. Otolaryng. **26**:170, 1937. Torrigiani, C. A.: Riv. oto-neuro-oftal. **1**:265, 1924.

2. (*a*) Alpers, B. J.: Ann. Surg. **101**:27, 1935. (*b*) Wolf, A., and Echlin, F.: Bull. Neurol. Inst. New York **5**:515, 1936. (*c*) Cushing, H., and Eisenhart, L.: Meningiomas, Springfield, Ill., Charles C Thomas, Publisher, 1938. (*d*) Siris, J. H., and Angrist, A.: Am. J. Surg. **57**:162, 1942. (*e*) Freiman, I. S., and Ficarra, B. J.: Arch. Path. **35**:900, 1943.

3. Chorobski, J.; Jarzymski, J., and Ferens, E.: Surg., Gynec. & Obst. **68**:677, 1939.

4. deBusscher, J.: J. belge de neurol. et de psychiat. **39**:81, 1939.

5. Hirschfeld, L.: Mém. de la Soc. biol. **3**:94, 1851.

6. Levitt, J. M.: Arch. Ophth. **12**:877, 1934.

7. Paleari, A.: Riv. oto-neuro-oftal. **15**:59, 1938.

8. Green, M. I., and Childrey, J. H.: J. Nerv. & Ment. Dis. **89**:650, 1939.

9. List, F.: Surg., Gynec. & Obst. **76**:480, 1943.

mass in the order of probabilities would be (1) calcified hemangioma, (2) calcified epidermoid cyst, (3) osteochondroma."

Moderate headache persisted unchanged until Feb. 1, 1943, when the pain suddenly became much more intense. Within a few hours the patient lapsed into coma, with generalized convulsions and clonic movements, more violent on the left. Lumbar puncture revealed grossly bloody fluid under an initial pressure of 30 mm. of mercury. The deep reflexes were exaggerated, more so on the left. Babinski's sign was present bilaterally. A diagnosis of subarachnoid hemorrhage was made. The patient died that night, after his temperature had risen to 106 F.

At autopsy, apart from the intracranial findings, there was situated at the pyloric ring a punched-out ulcer, 4 mm. in diameter and about 3 mm. deep, with an indurated, thickened base. A shallow superficial 3 mm. ulcer was situated proximally, almost adjacent to this lesion. The duodenal mucosa was intact.

The brain was tense and the convolutions were flattened. No blood was present over the convexity. The bony mass at the base of the skull projected upward

Fig. 1.—Lateral roentgenogram of skull (United States Army Medical Museum Negative 74122).

into the brain substance, rendering removal of the brain slightly difficult. A large amount of clotted blood was present around the tumor mass, especially on the left side, continuous with clotted blood extending upward into the cerebral defect. At the base of the brain, the tuber cinereum and the mamillary bodies were destroyed, and the cavity of the third ventricle was exposed, filled with clotted blood. The optic nerves, chiasm and tracts were completely spared, the defect occupying quite neatly the space bounded by the chiasm and the optic tracts. The chiasm was displaced forward and to the left, while the optic tracts were spread laterally. The circle of Willis could not be identified.

The tumor mass on the floor of the skull was about 3.5 cm. in height and averaged 3 cm. in diameter. It was irregularly nodular and bosselated, bony in consistency, with irregular zones of pearly gray appearance. Shreds of brain tissue, together with much fresh blood, dotted the surface. The mass covered the sella turcica but arose principally on the right side, where it extended over part of the greater wing of the sphenoid bone but did not involve the foramen rotundum. The foramen lacerum medium on the right was irregular and slightly enlarged, but the carotid artery appeared normal as it passed through the foramen. The middle fossa on the left side was completely spared. The entire bony mass, together with the sella turcica, was removed.

On coronal section of the fixed brain, both lateral ventricles were dilated and filled with clotted blood, but the cortex and the centrum ovale were not unusual. At the level of the third ventricle an irregular ragged defect replaced most of the hypothalamus. The defect, most prominent on the right, extended upward to include the most ventral portions of both thalami and laterally to include portions of the substantia nigra. The capsular fibers coursing down to form the peduncles appeared bilaterally compressed. The red nucleus was entirely spared. At lower levels the right pyramid appeared smaller than the left and the fourth ventricle contained clotted blood.

The tumor consisted of masses of cartilage intermingled with bone trabeculae. The cartilaginous elements, which generally were present in lobular formation, exhibited a variety of forms. Some lobules presented a well differentiated, light blue–staining clear matrix, within which were evenly distributed cartilage lacunae containing plump cells. Only rarely were binucleated forms seen, and the nuclei were of small size and regular appearance. In some lobules the matrix was loose and fibrillar, with an edematous and myxomatous appearance, while lacunar outlines were no longer visible. The individual cells were stellate, with the processes fading away into the myxoid ground substance. Still other lobules were more cellular, with distinct spindle-like and stellate cytoplasm merging into a syncytial mass of mesenchymal appearance. These various types of tissue were sometimes all present within a single lobule.

The bony portions of the tumor for the most part took the form of well modeled trabeculae with distinct lamellation. In the large and coarse trabeculae cement lines were numerous. Vascular channels comparable to haversian canals were present in these large trabeculae. Around such canals the lamellas tended to form concentric rings. Especially between the more delicate trabeculae there was abundant hemopoietic tissue of essentially normal cellular constitution.

The bone elements and the cartilage islands were intimately intermingled. Some cartilage islands, especially those near the surface, were surmounted by a rim of bone, which, in turn, was separated from adherent portions of brain tissue by a dense but narrow layer of fibrous tissue. Rarely were a few osteoblasts visible, adjacent to this fibrous layer. The osteoblasts were surrounded by minute zones of osteoid tissue. These portions of the tumor presented a reversal of the usual relationships obtaining in osteochondromas, in which as a rule the cartilaginous portions lie superficial to the bone elements.

In the deeper portions of the tumor were cartilage islands of varying size surrounded by masses of bone. The cartilage at the point of junction was generally calcified. These central foci of cartilage were responsible for the annular zones of diminished density in the roentgenogram, giving the radiologist the impression of calcified blood vessels. In the deeper portions of the tissue osteoblastic activity was almost entirely absent. Only a very rare trabecula exhibited a marginal row of osteoblasts.

In addition to the well formed bony and cartilaginous portions, there were large portions composed of calcified cartilage matrix with few cells. This matrix was broken up by strands of dense connective tissue that were poorly vascularized. The matrix was thus sculp-

tured into an appearance of coarse trabeculae, in which, however, there was a practical absence of marginal osteoblasts. At the junctions of the matrix with the connective tissue strands there was an especial concentration of calcium granules. A few multinucleated cells suggestive of osteoclasts were seen. Deep within the calcified matrix and with no relation to connective tissue there were scattered single dark-staining nuclei

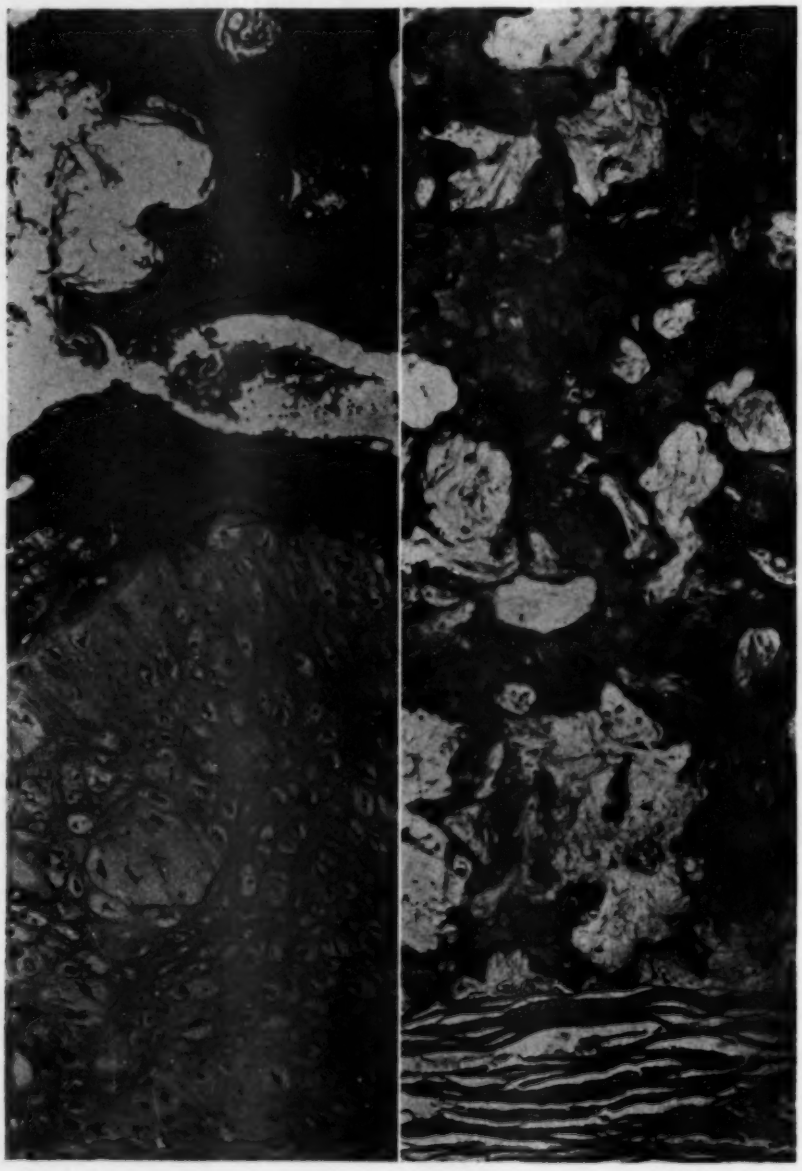

Fig. 2.—Left: osteochondroma showing mature cartilage and well formed bone (United States Army Medical Museum Negative 75617).

Right: Calcified cartilage matrix with transformation into bone (United States Army Medical Museum Negative 75616).

surrounded by a narrow zone of red-staining osteoid material, which contrasted vividly with the lumpy blue matrix. Such cells are interpreted as evidence of direct metaplasia of cartilage into bone.

Sections of the brain passing through the margin of the defect caused by the bony growth showed surprisingly little alteration. The margins had a frayed fibrillar appearance, with disintegration of the ground substance. But within the frayed and granular portions glial nuclei were present in essentially normal numbers. Small numbers of fresh red cells were present around the edges. Occasionally, small groups and clusters of compound granular corpuscles were seen at the edges, but they did not extend into the better preserved brain tissue. There were no zones of gliosis nor any fibrous leukocytic reaction. Nor were there any perivascular cuffings. Some altered glial cells appeared like astrocytes in the stage of clasmatodendrosis. Occasional neurons were seen close to the marginal defect. Although somewhat compressed and flattened, they were otherwise well preserved. Sections including ependymal lining of the involved third ventricle showed the lining normal. No alteration of nerve tissue at a distance from the defect was detected. It was not

feasible to make serial sections of the brain to determine in topographic relation what hypothalamic nuclei were involved.

Frozen sections of the brain stem were prepared for the study of the pyramidal tract. The pyramids, although apparently unequal in size, revealed no degeneration in sections stained for fat. No gliosis was present, and myelin sheaths appeared normal.

Sections of the pituitary gland revealed some excess of basophils, which displayed prominent granulations and occasional small vacuoles. No other changes were noted.

COMMENT

In comparison with similar cases reported in the literature the present case is unusual in that neurologic symptoms were not displayed until terminally. Mild headache, occurring only in the last few weeks of life, directed attention to the possibility of sinus infection. Routine roentgen examination of the sinuses accidentally revealed the tumor. In spite of the location of the tumor in the region of the chiasm, the fundi, the visual fields and the eye movements were entirely normal. The growth occupied the space bounded by the optic chiasm and the optic tracts, but these structures revealed no impingement. Death was due to a subarachnoid hemorrhage. The source of the bleeding was not discovered at autopsy.

The tumor was clearly of many years' standing and except for location was entirely benign. That the nerves of the base of the brain were not damaged is a purely fortuitous circumstance. An interesting speculation arises as to whether the peptic ulcer was not an indirect consequence of the tumor through the mechanism of hypothalamic damage. But this problem must remain in the speculative stage.

The cytologic reaction of the brain to the tumor is of interest. Rare compound granular corpuscles attested the minimal degree of acute destruction. Degeneration of astrocytes, rather than proliferation or progressive change, was the significant glial reaction. Gliosis, fibrosis and inflammation were equally lacking. No degeneration could be demonstrated in the pyramidal tracts, nor were there any clinical signs pointing to such involvement.

The predilection of this class of cartilaginous tumors for the sphenoid bone yields as yet no explanation.

SUMMARY

A 25 year old man experienced intermittent peptic ulcer for five years. While hospitalized for treatment of his latest attack, he complained of vague headache. About four weeks after the onset of the headache, he suddenly collapsed and died with signs of cerebral (subarachnoid) hemorrhage. Neurologic signs were absent except terminally. At autopsy a calcified mass at the base of the skull, previously visualized by roentgenogram, proved to be a large tumor of the sphenoid bone. It was diagnosed as osteochondroma.

MESOTHELIOMA OF THE PLEURA

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There can be little doubt that tumors may arise primarily in serous membranes of body cavities. McDonald,[1] Dick [2] and Klemperer and Rabin [3] have described undoubted examples of this. Young [4] by experimental injection of sudan III and olive oil showed that the serous lining of the pleural cavity may undergo metaplastic changes simulating carcinoma, while others (Birch-Hirschfeld; Glaser and Roloff [5]) have observed a close relationship between serous endothelium and fibroblasts. The changes of serous cells into fibroblasts and of fibroblasts into serous endothelium were traced in artificial adhesions by the latter workers.

Reports of cases of primary cancer of a serous membrane are rare, and some of those appearing in the literature are open to question. The latter fact was emphasized by Robertson [6] and Willis.[7] Both of these authors contended that a painstaking complete autopsy must be made to exclude a primary tumor elsewhere. This is particularly true when the diagnosis is bronchogenic carcinoma, and even more so when a diagnosis of mesothelial tumor is made merely on biopsies as was the case in the reports of Gömöri [8] and Banyai and Grill.[9] The best index of the occurrence of these tumors is to be obtained from a review of autopsy material. At the Toronto General Hospital pleural mesothelioma has been encountered in 7 of 7,878 consecutive autopsies covering a twenty year period (1923 to 1942). No record of a primary peritoneal or pericardial tumor has been found in our files.

I shall now report a case of an undoubted primary pleural tumor showing some features of special interest.

REPORT OF A CASE

A 25 year old woman admitted to Professor Duncan Graham's service was entirely well until July 1939, when she began to suffer a feeling of lassitude, and in September of the same year she noticed pain over and behind the left shoulder. Six months later pain developed in the left side of the chest anteriorly. This was not affected by breathing, and there was no associated cough, raising of sputum or dyspnea. On her admission to the hospital in January 1940 the important physical findings were confined to the chest. The mediastinum was shifted to the right, and the left side of the chest moved little with respiration. Vocal fremitus on the left side was absent, and posteriorly the percussion note was flat from the apex to the base of the lung. Breath sounds over the involved side were bronchial in character. There was marked clubbing of the fingers and the toes. On a number of occasions clear yellow fluid was aspirated from the left side of the chest. This contained neither tubercle bacilli nor tumor cells. Repeated thoracic taps later in the illness yielded only small quantities of light brown fluid, and solid particles persisted in plugging the aspirating needle. Bronchoscopy revealed no mucosal lesions other than redness and some narrowing of the orifice of the bronchus to the left upper lobe. Dyspnea, not present at the onset of the disease, became progressively more disturbing, and the patient died of respiratory failure in August 1942.

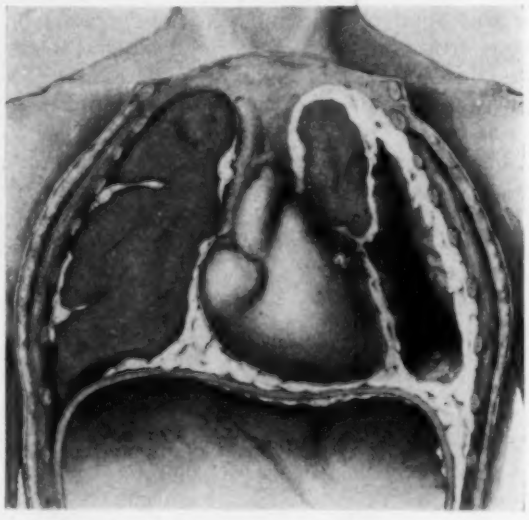

Fig. 1.—Distribution of the tumor in the thorax. Note the spread of tumor by continuity along the diaphragm.

Necropsy.—The main gross changes were found in the left pleural cavity. The left lung was compressed and atelectatic. It was entirely surrounded and encased by a thick, firm, gray sheet of tumor, which displaced it upward and medially. The demarcation between tumor and lung was sharp but of irregular outline. The tumor mass then curved around to cover the entire parietal surface of the left wall of the chest, to which it was densely adherent, so that the parietal pleura could not be seen. The tumor was also adherent to the diaphragm and the pericardium. A large cavity, presumably the left pleural space, was outlined by these two layers of

From the Department of Pathology, University of Toronto.

1. McDonald, S., Jr.: J. Path. & Bact. **43**:137, 1936.
2. Dick, J. C.: J. Path. & Bact. **47**:43, 1938.
3. Klemperer, P., and Rabin, C. B.: Arch. Path. **11**:385, 1931.
4. Young, J. S.: J. Path. & Bact. **31**:265, 1928.
5. Cited by Ewing, J.: Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1940, p. 337.
6. Robertson, H. E.: J. Cancer Research **8**:317, 1924.
7. Willis, R. A.: J. Path. & Bact. **47**:35, 1938.
8. Gömöri, G.: Am. J. Surg. **33**:150, 1936.
9. Banyai, A. L., and Grill, J.: Am. Rev. Tuberc. **27**:193, 1933.

tumor (fig. 1). The space contained thick, dirty brown fluid and was lined by a roughened, granular surface. The cut surface of the tumor, the consistency of which was almost cartilaginous, was gray-white and glistening, and presented scattered pinhead-sized cystic areas containing thin mucoid material. The tumor had spread into the parenchyma of the left lung at several points throughout the periphery, for a distance of 0.5 to 1 cm. in the form of a plaque measuring 2 cm. in diameter and was slightly elevated above the surface. Apart from this, the remainder of the pericardial surface was smooth and glistening. Secondary metastases, ranging from 0.5 to 1.5 cm. in thickness, also invaded the pleural and peritoneal surfaces of both halves of the diaphragm. The tumor over the diaphragm presented a peculiar reticulated pattern of growth and formed flattened,

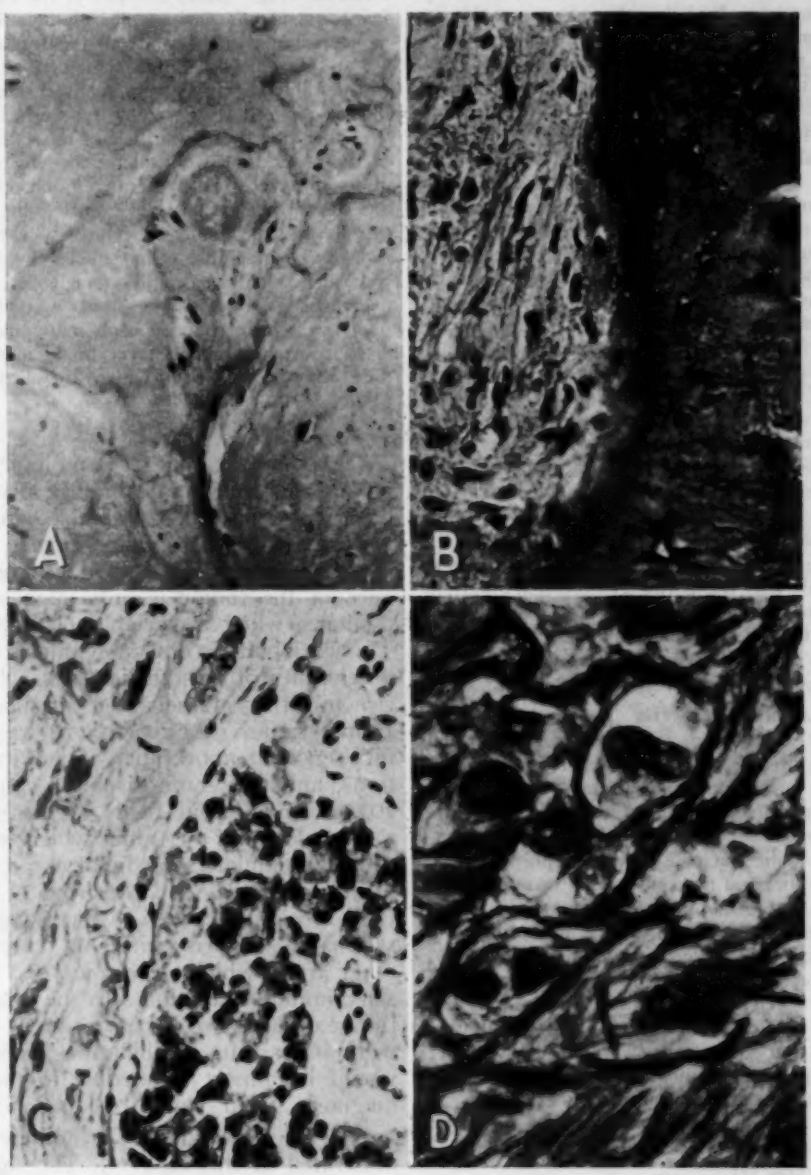

Fig. 2.—*A*, a section of the primary tumor showing the whorled pattern and hyaline appearance. The stroma is prominent, and only scattered nuclei are seen. Hematoxylin and eosin; × 270.

B, osteoid matrix. This occupies the right half of the photograph. A narrow calcific line separates it from the adjacent soft tissue tumor. Hematoxylin and eosin; × 360.

C, area in which the tumor mimics scirrhous carcinoma. Note the clear definition of the tumor cells and the amount of stroma and compare with *A*. Hematoxylin and eosin; × 360.

D, reticulum encasing each cell. Several cells in the lower half of the photograph contain reticulum fibrils in the cytoplasm. Laidlaw silver stain; × 930.

The parietal pericardium over the left auricle was involved by the tumor, and a secondary metastatic nodule was found beneath the visceral pericardium. This was plaquelike lesions which were, for the most part, confluent. This was so marked on the peritoneal side that it resembled a trabeculated urinary bladder.

The right pleural cavity contained 900 cc. of clear straw-colored fluid, and the visceral pleura of the right lung, from apex to base, presented small plaques of tumor growth. These ranged from 0.3 to 1.2 cm. in diameter and were discrete, firm and gray-white. These plaque-like lesions also extended along the interlobar fissure, between the middle and lower lobes, to the root of the lung, where they surrounded the hilar portion of the right pulmonary artery. Several bronchopulmonary lymph nodes were involved by secondary neoplasms. A few small tumor nodules, all of uniform size, were found scattered through the parenchyma of the right lung. The remainder of the right lung showed congestion and edema. A careful search disclosed no evidence of tumor in the tracheobronchial tree.

The abdominal cavity contained 250 cc. of clear yellow fluid, and both the parietal and the visceral layer of the peritoneum were studded with secondary tumor deposits. Most of these were distributed in the form of nodules along the course of lymphatic channels beneath the serosal surface of the gastrointestinal tract. The nodules measured from 1 to 2 cm. in diameter, and the involved lymphatics appeared as gray cordlike streaks. The mucosal surface of the entire gastrointestinal tract was normal. Tumor deposits were found in the mesentery of the small intestine and in the gastrocolic omentum. The liver was enlarged, weighing 1,725 Gm., and contained a number of similar lesions. These were situated along the periphery, just beneath the capsule, and all of them were of a cartilaginous consistency. The cut surface of the hepatic nodules revealed tiny clear cystlike structures.

The breasts, the gallbladder, the pancreas, the spleen, the adrenal glands and the entire genitourinary system presented no abnormalities. No lesions were found in the mouth, the tongue, the pharynx, the larynx, the thyroid gland or other structures of the neck. The brain and the osseous system also were normal.

Microscopic Examination of the Tumor.—Histologically the pleural tumor was relatively acellular and avascular. In scattered areas, particularly about the periphery of the tumor, nuclei of varying shapes were seen. Some were rounded while others were fusiform. Some of the rounded nuclei were pale staining and contained small basophilic nucleoli. The cytoplasm of the cells was eosinophilic, and the cell outline was indistinct and merged into the eosinophilic fibrillar stroma. The fibrils were compactly arranged in a whorled fashion and gave a peculiar appearance of hyalinosis (fig. 2 *A*). Only an occasional elongated, markedly compressed nucleus could be seen in the central portions of the tumor, where the stroma predominated. Mitotic figures were infrequent, and giant cells were absent. Signet ring cells were present in moderate numbers. Mucicarmine stains gave negative results on these cells. A connective tissue stain showed fibrillar stroma arranged in an interlacing and whorled pattern. Sudan IV stains failed to reveal fat.

An unexpected finding in these sections was an atypical osteoid matrix in the central portions of the tumor (fig. 2 *B*). This was surrounded by a narrow zone of calcium and stained more deeply than the fibrillar tumor stroma. The adjacent lung parenchyma was markedly compressed, and the alveoli were almost completely obliterated. Invasion of the lung substance by tumor had occurred in several areas. The microscopic picture of the hepatic nodules resembled that of the pleural tumor. Here, however, the interlacing and whorled pattern was decidedly more evident, and signet ring cells, which again gave negative results with mucicarmine, were more numerous. No osteoid matrix was found in these sections. Here again the tumor had a tendency toward overproduction of connective tissue, which gave it a hyaline appearance.

In the right lung the tumor in some sections was desmoplastic and relatively acellular. An overabundance of homogeneously eosinophilic stroma was present. Again atypical osteoid matrix resembling that found in the primary pleural tumor was seen surrounded by a calcific ring. Other sections of the right lung showed involvement of perivascular lymphatics, bronchopulmonary lymph nodes and portions of lung parenchyma by an extremely cellular tumor. The cells presented considerable variation from each other and were divided into two definite types. One type was fusiform whereas the other had the appearance of an epithelial cell. Cells of the latter type were surrounded by varying amounts of stroma, were loosely arranged and polyhedral. Their cell membrane was well defined (fig. 2 *C*), and the cytoplasm was abundant, relatively clear and homogeneously eosinophilic. The nuclei were vesicular and contained small basophilic nucleoli. The tumor, as demonstrated by a silver stain, was rich in reticulum fibers, which surrounded each individual cell. In some areas short, thin fibrils were found in the cytoplasm of the tumor cells, from which they appeared to arise (fig. 2 *D*).

COMMENT

It is evident that a pathologic diagnosis of pleural mesothelioma cannot be made on any one criterion. The most valuable single procedure is a thorough gross examination to rule out other possible primary sites. The microscopic appearance of the tumor is only suggestive of mesothelioma when a variable picture is observed. This variability is obviously due to the variegated potentiality of mesothelial cells, which may give rise to either epithelial or fibroblastic elements. In the tumor described there was considerable difference in appearance found in various sections, and this same disparity could be demonstrated in adjacent fields of the same block of tissue. Parts of the tumor which were extremely cellular simulated medullary carcinoma, while other portions which consisted of cords of epithelial cells separated by varying amounts of stroma resembled scirrhous carcinoma. Still other areas, which were composed of fibroblastic cells, imitated sarcoma. In the final analysis the tumor consisted of two main types of cells which were in various combinations, and therefore it would be almost impossible to make a correct diagnosis from study of one individual slide.

Several other distinct histologic features are illustrated by this case: 1. The signet ring cells were most numerous in the acellular or collagenous portions of the tumor and absent in the younger, more actively growing and cellular areas, where the stroma was at a minimum. They failed to stain for fat and also were negative with mucicarmine stains. Thus it is most probable that these cells were the result of a degenerative change rather than the result of a secretory function. This is contrary to Zeckwer's [10] opinion, although she did not mention

10. Zeckwer, I. T.: Arch. Int. Med. **34**:191, 1924.

mucicarmine stains in her description. 2. The tumor was rich in reticulum, and, in addition to this general increase, argentaffin fibrils surrounded individual tumor cells, to which they were intimately related. The cytoplasm of some of the cells was made up of similar fibrils, and it appeared that these were converted into extracellular reticulum. Other authors [11] have reported reticulum intimately associated with the tumor cells, but they failed to state whether or not the cells actually gave rise to the fibrils. Foot and Day [12] stated that mesothelioma has abundant reticulum between its cells but that fibrils are lacking in the cytoplasm. They held that reticulum is formed not as the result of cytoplasmic transformation but by preexisting fibers in the intercellular fluids. The observations in the present case are not in accord with the foregoing statement. 3. The most striking feature of the tumor observed by me was the presence of osteoid matrix. This was found only on microscopic study and could not be detected on naked eye examination of the gross material. Osteoid matrix, to my knowledge, has not hitherto been reported in any case of mesothelioma of the pleura. This substance was present in the primary growth and also in a secondary nodule in the opposite lung. From this observation it can be said that bone formation in this case was not the result of local conditions in the pleura but was an inherent property of the tumor. This is still in keeping with the potentialities of mesothelial cells to give rise to either epithelial or mesenchymal elements.

Histologic features such as giant cells, lipoid storage cells and lymphocyte-like cells, which were recorded by other writers,[13] were not found in this case.

11. Klemperer, P., and Tedeschi, C.: J. Mt. Sinai Hosp. **8**:710, 1942. Klemperer and Rabin.[3]

12. Foot, N. C., and Day, H. A.: Am. J. Path. **1**:431, 1925.

SUMMARY

Analysis of the observations in previously recorded cases of mesothelioma of the pleura reveals no mention of the presence of an osteoid matrix, yet this was the most striking feature in the mesothelioma of the pleura described here, which in most other respects corresponded to the earlier described examples.

13. DuBray, E. S., and Rosson, F. B.: Arch. Int. Med. **26**:715, 1920. Klemperer and Rabin.[3] Zeckwer.[10] Klemperer and Tedeschi.[11]

CARCINOMA OF THE LUNG IN A TEN YEAR OLD NEGRO BOY

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The lung is one of the rarest sites for primary cancer in childhood. Less than 10 cases of primary pulmonary cancer have been reported as occurring in the first decade of life.[1] Among 209 subjects with carcinoma of the lung recently surveyed,[2] the youngest was 21, and only 2 were in the third decade of life. In Simons'[3] tabulation of 2,796 cases of carcinoma of the lung 1 was listed as having occurred before the age of 9 years, and 6, between the ages of 10 and 19 years; Ochsner and DeBakey[4] listed among 4,307 cases 7 and 31 in the same age groups respectively. The case herein recorded occurred in a Negro child, probably the second such case ever observed. It presented a complicated clinical problem because of the unusual cellular structure of the growth and the mode and the extent of its spread.

REPORT OF A CASE

A 10 year old Negro boy was admitted to the University Hospitals in Oklahoma City, Oct. 9, 1942, with complaints of increasing weakness of both legs and pain in the lower part of the back for the past two months. This, he thought, began shortly after he lifted a piece of heavy scrap iron, and progressed until he was unable to stand without some support. The past and family histories were essentially without significance. Both parents, a sister of 16 and three brothers 15, 13 and 8 years old were living and well.

The child seemed well developed, well nourished and in no apparent discomfort. He could stand without support only for a minute or two and could raise neither leg when lying on his back. The right patellar and both achilles tendon reflexes were absent. Raising either leg straight caused pain in the back. There was bilateral costovertebral tenderness. The lungs seemed clear on percussion and auscultation. The right border of the heart extended 3 cm. to the right of the midsternal line in the fourth interspace; the apex was 7 cm. to the left of the midsternal line in the fifth interspace. A thrill was felt over the apex, and a loud systolic murmur was audible over the entire cardiac area; P-2 was louder than A-2. There was no incontinence of urine or of feces.

Roentgenographic examination of the chest revealed a moderate increase in size of the cardiac shadow and an accentuation of the hilar vascular markings of the lungs; the right interlobar septum was broad; the costophrenic angles were free. The left fifth rib was broadened and appeared dense; its pattern was almost completely obliterated. Further examination, which eventually included the entire skeleton, revealed similar involvement, with broadening of the periosteum and widening of the cortex, of the following parts of the skeleton: the bodies of the eleventh and twelfth dorsal and first lumbar vertebrae, including their transverse and spinous processes; the left transverse process of the second lumbar vertebra and the right of the fourth lumbar vertebra; the right ilium, including the acetabulum; the sacral portion of the left ilium; the neck of the left femur and the distal ends (diaphyses and epiphyses) of both femurs; the proximal and distal ends of both tibias; the spinous and coracoid processes and the vertebral portion of the left scapula; the proximal ends of both humeri and the distal end of the right ulna. The short bones of the hands and of the feet and the skull were not involved (fig. 1).

The urine was acid, with a specific gravity of 1.032; it revealed no albumin and no sugar. The red blood cell count was 4,370,000; the hemoglobin content was 12 Gm.; the white blood cell count was 8,200, with polymorphonuclears 56, metamyelocytes 3, lymphocytes 32, eosinophils 7 and monocytes 2 per cent. The Kahn test was negative. The cerebrospinal fluid showed a protein content of 500 mg. per hundred cubic centimeters; the colloidal gold curve was 0000000011; the Wassermann reaction was negative. The nonprotein nitrogen in the blood amounted to 25 mg., the blood calcium to 10.4 mg. and the blood phosphorus to 2.8 mg. per hundred cubic centimeters; the phosphatase value was 8.8 units (Bodansky). In the Westergren sedimentation test the cells settled to 41.5 per cent in one hour with a diagonal line. A hemogram failed to reveal sickle cells. The urine did not contain Bence Jones protein. On March 24, 1943 the blood calcium was 11 mg. and the blood phosphorus 1.6 mg. per hundred cubic centimeters; the phosphatase value was 29.12 units (Bodansky). The total serum protein amounted to 7.2 Gm., the albumin to 4.8 Gm. and the globulin to 2.4 Gm. per hundred cubic centimeters.

Soon after admission incontinence of urine and feces and anesthesia of the feet and legs developed. On October 18, nine days after admission, a laminectomy (Dr. Harry Wilkins) at the level of the eleventh dorsal to third lumbar vertebra disclosed a neoplastic involvement of the spinal column with invasion of the spinal canal. Microscopic examination revealed the growth to be composed of irregular nests of spindle-shaped cells with deeply stained nuclei and no discernible cytoplasmic borders. Newly formed fibrous connective tissue stroma seemed to surround the tumor cell nests which invaded and replaced striated muscle bundles. The individual and group characteristics of the cells suggested that they were of epithelial origin.

Following the operation, there was some transient improvement in the anesthesia, and some power of movement returned in the lower extremities. Roentgen irradiation of the proximal third of the right humerus was given over a ten day period with no demonstrable effect (total dose 2,000 r, with one port, 8 by 10 cm.; distance 50 cm.; 200 kilovolts; 20 milliamperes; Thoraeus filter).

From the Laboratories of the University Hospitals and the Department of Radiology, University of Oklahoma School of Medicine.

1. Hirsch, E. F., and Ryerson, E. W.: Arch. Surg. **16**:1, 1928. Beardsley, J. M.: Canad. M. A. J. **29**: 257, 1933. Gould, L. K.: J. Indiana M. A. **27**:332, 1934. Hauser, H.: Radiology **39**:33, 1942. Field, C. E., and Quilliam, J. P.: Brit. M. J. **1**:691, 1943.

2. Halpert, B.: J. A. M. A. **117**:1090, 1941; Cancer Research **1**:900, 1941.

3. Simons, E. J.: Primary Carcinoma of the Lung, Chicago, Year Book Publishers, Inc., 1937.

4. Ochsner, A., and DeBakey, M.: Arch. Surg. **42**:209, 1941.

On December 10 intravenous pyelograms disclosed good secretory function of both kidneys with normal configuration of the calices, pelves and ureters. Roentgenographic examination of the chest on December 17 revealed a partial pneumothorax, moderate infiltration of the right upper lung field and an increase of the hilar vascular markings. Extension of the skeletal lesion in the dorsolumbar region was noted.

On March 9, 1943 a biopsy (Dr. Willis K. West) of the left femur disclosed neoplastic involvement similar to that seen in the tissue removed at laminectomy. No enlargement of any of the superficial lymph nodes was noted on repeated examination.

The patient's general condition became gradually worse; progressive secondary anemia, fever and hematuria developed, and the patient died on April 7, after about six months' stay in the hospital.

Necropsy (nine hours after death).—The body was 144 cm. long and weighed approximately 70 pounds (32 Kg.). The superficial lymph nodes were not palpably enlarged. No change was noted in the mouth or the pharynx. The neck was symmetric, with no involvement of the soft parts. The supraclavicular fossae were sunken. The chest was flat. The abdomen was prominent. The scrotum and the penis were swollen and pitted on pressure. There was a linear scar 15 cm. long about the lower thoracic and lumbar regions, and an ulcer 7 by 4 cm. over the sacrum. There were similar but smaller denuded areas of skin over the right iliac crest, the right knee, the crest of the left ilium and the left medial malleolus. The musculature of the upper and lower extremities appeared wasted.

No changes were noted in the peritoneal cavity. The right pleural cavity was partly obliterated by easily torn adhesions and contained 200 cc. of pale straw-colored fluid. The surfaces of the lung were studded with gray-white nodules from 0.5 to 1 cm. in diameter (fig. 2). The left pleural cavity resembled the right, with some nodules present also in the mediastinal portion of the parietal pleura. The pericardial cavity contained 80 cc. of pale straw-colored fluid. There were no anomalies of the large vessels or of the tributaries of the coronary sinus. The heart measured 11 cm. from base to apex and 10 cm. across the base. On the posterior surface there was a gray-white area 2 cm. in diameter. From this a conical mass, 6 by 3 cm., protruded into the lumen of the left ventricle, replacing a papillary muscle. No other changes were noted in the chambers of the heart or about the orifices and valves. The heart weighed 250 Gm.

The tracheobronchial lymph nodes were matted together to a mass measuring 6 by 4 cm. The esophagus with its wall intact was bent over this mass. There was also a mass anteriorly and to the left of the trachea, 6 by 5 cm. These were composed mostly of neoplastic tissue similar to that of the nodules protruding from the surfaces of the lungs (fig. 2). In the branch of the right bronchus leading to the middle lobe there was a thickening in the wall for a length of 1 cm. Underneath this and continuous with it was a mass 8 by 6 cm. This was the largest area of involvement in the lung and the only one connected with a bronchial branch. Other nodules were few and mostly confined to the periphery of the lungs. The pleural surfaces were studded with various-sized nodules. The lungs together weighed 950 Gm. There was a neoplastic area 2 cm. in diameter in the upper pole of the right kidney, and there were three similar protruding nodules measuring 1, 2 and 3 cm., respectively, on the surface of the left kidney. No metastases were encountered in the liver or the adrenal glands or in any of the other viscera. There was a protruding mass at the level of the attachments of the left fourth and fifth ribs. Similar thickenings were seen over the left seventh and right tenth and eleventh ribs. The marrow

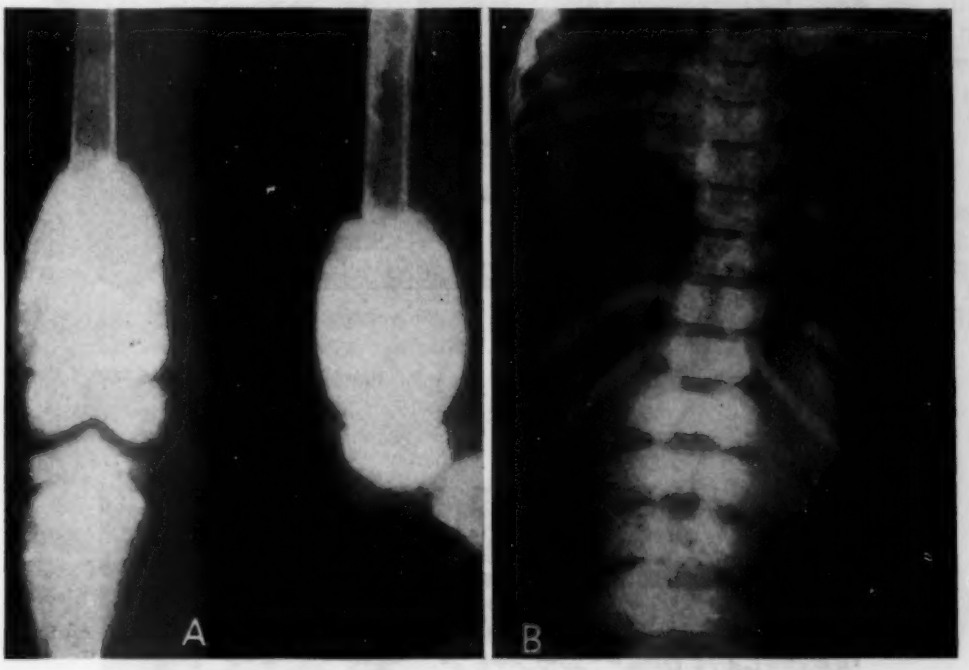


Fig. 1.—Roentgenographic appearance of (*A*) the right lower extremity and (*B*) the vertebral column (after laminectomy).

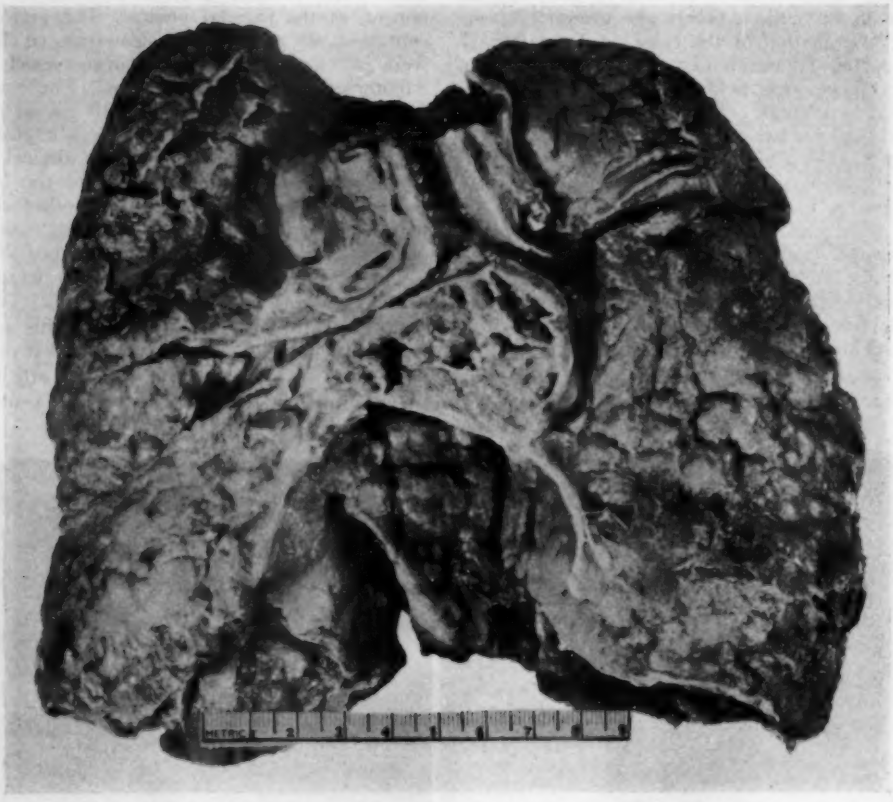


Fig. 2.—Posterior view of the lungs.

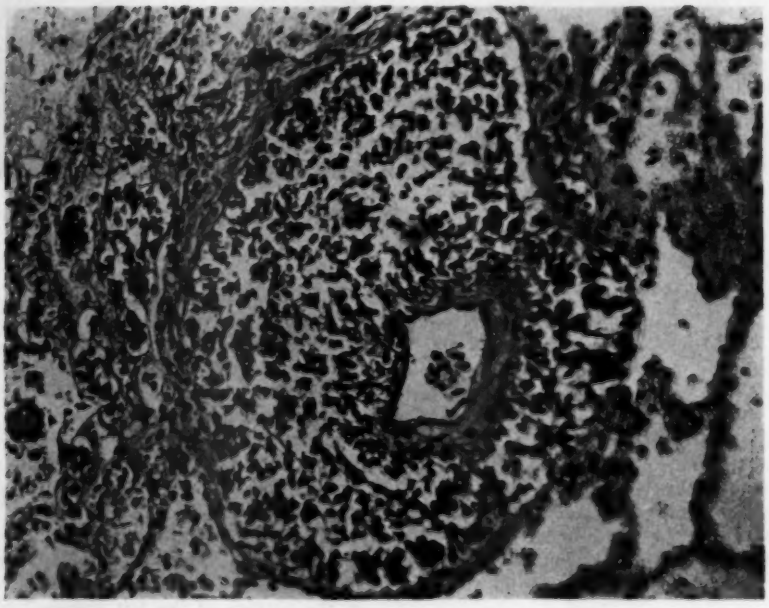


Fig. 3.—Microscopic appearance of the neoplasm in the lungs; × 120.

spaces of the bodies of the thoracic and lumbar vertebrae were replaced by gray-white neoplastic tissue. The anatomic diagnosis based on the observations described was carcinoma of the right lung with metastasis to the regional lymph nodes, the pleurae, the heart, the skeleton and the kidneys.

Microscopically, the mucosal surface of the bronchial branch leading to the right middle lobe was devoid of covering epithelium, and the tunica propria and all the layers beneath were infiltrated with sheets of neoplastic cells composed of round or elongated deeply stained nuclei having scanty cytoplasm with hardly discernible borders. These cells formed no particular pattern. The peripheral cells of the tumor cell nests in places had a palisade arrangement. The cells surrounded ducts and acini of the glands and filled lumens of atriums and alveoli and lymph channels about the blood vessels (fig. 3). In a tracheobronchial lymph node there was almost complete replacement of the lymphatic tissue by neoplastic cells and stroma similar to those in the lung. In the heart there were areas of infiltration and replacement of the myocardial fibers by nests of tumor cells. About the periphery of the growth the muscle fibers were delicate and pushed aside. In some fields there was complete replacement of all myocardial fibers by neoplastic cells and their scanty stroma. In the kidneys similar sheets of neoplastic cells with extensive areas of hemorrhage and necrosis invaded, replaced or spread apart the glomeruli and the convoluted, connecting and collecting tubules. The extension of the growth seemed to follow lymph channels about the blood vessels. In the osseous tissue involved, complete filling of the marrow spaces was seen over extensive areas with broadening of the bony lamellas and no necrosis. The individual and group characteristics of the neoplastic cells and their relation to invaded tissue were alike in all the involved sites, and the neoplasm was interpreted to be a reserve cell carcinoma of the lung.

COMMENT

According to Ochsner and DeBakey,[4] carcinoma of the lung metastasizes most frequently to regional lymph nodes, next most frequently to the liver, third, to the skeleton and fourth to the adrenal glands. In our case, skeletal involvement was the dominant feature, and there was no metastasis to either the liver or the adrenal glands.

The skeletal lesions produced by carcinoma of the lung are usually osteolytic, destroying bony structure and pattern. This growth seemed to be osteoplastic, filling the marrow spaces, which were separated by broad and intact lamellas, and broadening the periosteal zones. To determine whether this type of relation between osseous tissue and the invading neoplasm was due to the youth of the patient, to the nature of the growth or to the combination of the two requires further investigation.

SUMMARY

A reserve cell carcinoma of the lung occurred in a Negro boy 10 years old. Extensive osteoplastic skeletal lesions and their sequences dominated the clinical picture, which was further complicated by massive neoplastic involvement of the heart.

Laboratory Methods and Technical Notes

A RAPID TRICHROME STAIN

O. J. Pollak, M.D., Taunton, Mass.

Although the value of the various trichrome stains [1] is well known, the technical difficulties inherent in their use interfere with their adoption for routine work. Chief among these difficulties are (1) the number of times the sections must be transferred from one solution to the other and (2) the length of time required for the completion of the preparation.

The simplified trichrome stain recommended for the staining of vaginal smears [2] is not suitable for the staining of tissue sections. However, by imitating its formula but combining different [1e] trichrome stain ingredients in a single solution, an excellent polychromatic differentiation can be obtained with a very simple technic. Permanent slides are obtained following the use of any standard fixative.

STAINING SOLUTIONS

1. Weigert's iron-hematoxylin, fortified. Instead of 1.0 Gm. of hematoxylin as originally prescribed 1.25 Gm. per hundred cubic centimeters of 95 per cent alcohol is used.
2. Distilled water with 0.2 per cent of glacial acetic acid.
3. Trichrome mixture.[3] This is made up as follows:

Acid fuchsin	0.50 Gm.
Ponceau 2R	1.00 Gm.
Light green SF, yellowish	0.45 Gm.
Orange G	0.75 Gm.
Phosphotungstic acid, chemically pure	1.50 Gm.
Phosphomolybdic acid, chemically pure	1.50 Gm.
Glacial acetic acid	3.00 cc.
Ethyl alcohol, 50 per cent	up to 300.00 cc.

First, glacial acetic acid is added to the alcohol. Of this acidified alcohol, 50 cc. portions are prepared in four beakers: in the first beaker acid fuchsin and ponceau 2R are dissolved; in the second, light green; in the third, orange G and phosphotungstic acid, and in the fourth, phosphomolybdic acid. The rest of the acidified alcohol is used to wash out the beakers and to make up the volume. The dyes used are from the National Aniline & Chemical Company. All ingredients dissolve easily except for the phosphomolybdic acid, which will dissolve after slight warming of the beaker. The mixture is filtered and ready for use. It keeps well.

METHOD OF STAINING

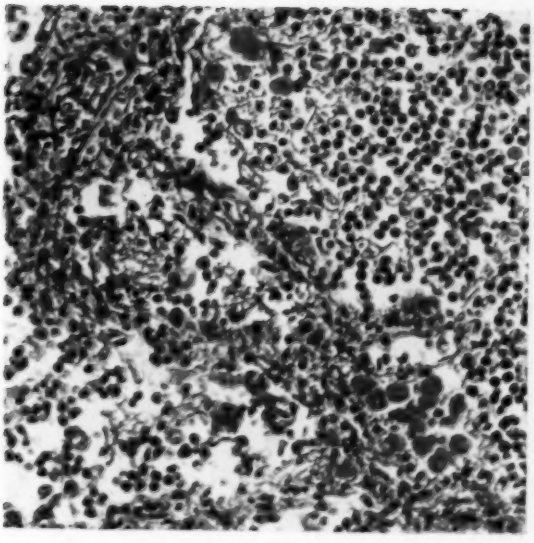
1. Stain in Weigert's iron-hematoxylin, fortified, for ten to twenty minutes.
2. Wash slides in running water for the same length of time.
3. Stain in the trichrome mixture for three to seven minutes and control with the microscope.
4. Differentiate shortly with acidified distilled water and control with the microscope.
5. Dehydrate quickly in 95 per cent alcohol followed by absolute alcohol, clear in xylene and mount in balsam.

RESULTS

Nuclei stain black. Collagen stains blue-green, muscle red, elastic fibers red and fibrin purple. Bone stains green, cartilage blue-green, calcium deep purple. Mucus stains blue-green, hyalin pale blue and colloid orange or light red. Keratin stains orange-red. Red blood corpuscles stain orange. Hemosiderin stains olive green and lipofuscin orange-brown, while anthracotic pigment appears black. Prostatic corpuscles stain blue-green, some with an orange-brown center. Neuroglia stains yellow-red, axis-cylinders pink and endoneurium and perineurium green.

From the Laboratories of Taunton State Hospital.

1. (*a*) Mallory, F. B.: J. Exper. Med. **5**:15, 1910. (*b*) Heidenhain, M.: Ztschr. f. wissensch. Mikr. **32**:361, 1915. (*c*) Masson, P.: J. Tech. Methods **12**:75, 1929. (*d*) Foot, N. C.: Stain Technol. **8**:101, 1933. (*e*) Goldner, J.: Am. J. Path. **14**:237, 1938.
2. Shorr, E.: Science **94**:545, 1941.
3. The trichrome mixture may be obtained from the Will Corporation, Rochester, N. Y.



Lobar pneumonia. Fixation in Zenker's formaldehyde solution (Zenker's solution made with solution of formaldehyde U. S. P. instead of acetic acid); paraffin section; trichrome stain; × 200.

UNIV. OF MICH.

Notes and News

Awards.—The gold-headed cane was awarded to Ludvig Hektoen by the American Association of Pathologists and Bacteriologists, April 8, 1944, in recognition of distinguished service to pathology and unselfish devotion to the highest ideals of the profession. Previous awards have been made to Harold C. Ernst, William H. Welch, Theobald Smith, Frank B. Mallory and James Ewing.

Alexander Lipschutz, director of the department of experimental medicine of the Chilean National Health Service at Santiago, has been selected to be the recipient of the second Charles L. Mayer $2,000 prize, which is administered by the National Science Fund of the National Academy of Sciences. The award was offered for "an outstanding contribution made in 1943 to present-day knowledge of factors affecting the growth of animal cells, with particular reference to human cancer."

Hattie Alexander, associate professor of pediatrics at Columbia University, is the recipient of the $500 prize given annually under the auspices of the American Academy of Pediatrics by Mead Johnson and Company for "outstanding scientific contributions to pediatrics in the United States." The award was made "in recognition of her work on the treatment of Haemophilus influenzae meningitis."

Deaths.—S. Oberndorfer, since 1933 professor of pathology in the University of Istanbul, Turkey, died March 1, 1944, at the age of 67, of pulmonary cancer. Until 1933 he had been for many years head of the department of pathology at the Schwabing Hospital, Munich, Germany. Many American students who visited Munich will recall his genial personality, his unusual experience and his ability as teacher. His research was successfully continued in Istanbul, particularly in the field of tumors. His contributions on intestinal tumors and diseases of the male genitalia, published in the Henke-Lubarsch Handbuch, are well known.

Randle C. Rosenberger, professor of immunology and bacteriology in Jefferson Medical College, Philadelphia, died February 21, aged 70, of myeloblastic leukemia.

John Eyre, consulting bacteriologist to Guy's Hospital and emeritus professor of bacteriology at the University of London, died February 7, at the age of 74.

Society News.—At a meeting of the Council of the American Association of Pathologists and Bacteriologists, held April 8, 1944, it was voted that unless unforeseen circumstances arise, the next annual scientific sessions of the association will be held at the University of Chicago, Friday and Saturday, May 4 and 5, 1945. The topic selected for the symposium is "Infectious Granulomas, Exclusive of Tuberculosis and Syphilis." Wiley D. Forbus, professor of pathology at Duke University, will be the referee.

Appointments.—George K. Strode, associate director of the International Health Division of the Rockefeller Foundation, in charge of the division's work in Europe, has been appointed director of the division to succeed Wilbur A. Sawyer, who will retire, effective September 1.

Major Irving Graef of the Medical Corps, Army of the United States, on leave of absence as associate professor of pathology at the New York University College of Medicine and as pathologist to the Bellevue Hospital, New York, has been appointed director of the medical research laboratory at Dugway Proving Ground, Tooele, Utah. This laboratory is an installation of the Medical Division of the Office of the Chief of Chemical Warfare Service.

New appointments to the full time faculty of Southwestern Medical College of the Southwestern Medical Foundation, Dallas, Texas, include:

Simon Edward Sulkin, formerly instructor in bacteriology and immunology at Washington University School of Medicine and director of the virus laboratory of the St. Louis Health Division, to the position of associate professor of bacteriology; Atticus J. Gill, formerly assistant professor of pathology at the University of Tennessee College of Medicine, Memphis, to the position of assistant in pathology; Robert Merrett Pike, formerly bacteriologist and assistant director of Bassett Laboratories, Cooperstown, N. Y., to the position of assistant professor of bacteriology.

Anderson Nettleship, passed assistant surgeon in the United States Public Health Service Reserve, Bethesda, Md., has been appointed associate professor of pathology at the University of Oklahoma School of Medicine, Oklahoma City.

Roy R. Kracke, professor of pathology and bacteriology and chairman of the department at Emory University School of Medicine, Atlanta, Ga., has been named dean of the new Medical College of Alabama to be organized in Birmingham.

Cecil A. Krakower, formerly with the Columbia University School of Tropical Medicine in San Juan, Puerto Rico, has been appointed assistant professor of bacteriology and pathology at Tulane University of Louisiana, New Orleans.

Kenneth L. Burdon, formerly assistant professor of immunology and bacteriology at Louisiana State University School of Medicine, New Orleans, was appointed professor of bacteriology at the Baylor University College of Medicine at Houston, Texas, recently.

Charles F. Branch, professor of pathology, Boston University School of Medicine, has been appointed dean of the school to succeed Bennett F. Avery, who resigned to become director general of public health of Iran.

Edgar M. Medlar has been appointed associate professor of pathology in the Columbia University College of Physicians and Surgeons.

Resignation.—Orin A. Ogilvie has resigned as professor of bacteriology and pathology at the University of Utah School of Medicine, Salt Lake City.

Books Received

THE RIDDLE OF CANCER. Charles Oberling, M.D. Translated by William H. Woglom, M.D. Pp. 196. Price $3. New Haven, Conn.: Yale University Press, 1944.

Oberling's "Le problème du Cancer" (1942) gives an excellent review of fundamental cancer research. It discusses clearly and thoroughly its theoretical and experimental phases, but it does not take up clinical cancer research except in a minor way in the part on hereditary influences. The last chapter is a cleverly conducted, but not unreasonable, argument in favor of the theory that cancer in all its forms is caused by virus. It is rather curious that the book hardly mentions the artificial culture of cancer cells and that it does not stress the fact or the significance of the irreversibility of the cancerized cell. Except for this omission there is perhaps no book in English that reviews as smoothly and clearly the present state of fundamental cancer research as Oberling's. Woglom's translation is excellent. The bibliography has been expanded and a needed good index has been added. It is an instructive book for the reader who has an intelligent interest in research and in cancer.

THE CONTROL OF CROSS INFECTION IN HOSPITALS. Medical Research Council War Memorandum no. 11. Pp. 34, with 6 illustrations. Price 6 pence. London: His Majesty's Stationery Office, 1944.

CORRECTION

In the article by Dr. Douglas Symmers entitled "Spindle and Giant Cell Sarcoma Arising from Unidentified Precordial Bodies" in the March issue (Arch. Path. **37**:180 [March] 1944), the legend for figure 1 *B* should read "upper right corner" instead of "lower left corner."